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NUTRITIONAL STUDIES OF THREE SPECIES OF GLŒOSPORIUM

I. Effect of Different Sources of Carbon and Some of Their Mixtures

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Introduction

It has long been established that carbon as well as a number of other elements are indispensable for the growth of fungi. Grewal (1954) has given a summarized account of the effect of different sources of carbon on the growth and sporulation of various fungi. A survey of literature clearly indicates that all the micro-organisms do not grow equally well on any single source of carbon and the best source for any organism has to be determined.

In nature the fungi usually find mixed sources of carbon. Horr (1936) investigated the growth of *Aspergillus niger* on a mixture of glucose and galactose. He found that galactose alone was unsatisfactory for the growth of *A. niger* but it could be utilized satisfactorily from the mixture. Steinberg (1939) also studied the effect of mixed carbon sources on the growth of *A. niger*. He found that a combination of various sources of carbon (*e.g.*, mannitol and lactose) supported more growth while others (*viz.*, glycerol and galactose) gave less growth than it could develop on any of them used singly. An attempt has, therefore, been made to find out the best source of carbon for three different species of Glœosporium and to determine the effect of mixtures containing different sources of carbon.

Material and Method

The three fungi *Glœosporium psidii* Delacr., *Glœosporium limetticolum* Claus. and *Glœosporium citricolum* Speg. were isolated from *Psidium gujava*, *Citrus aurantifolia* and *Citrus medica* respectively. The isolation and purification technique have already been described by the authors in their previous paper (1954). Five grams of glucose present in Asthana and Hawker's medium A* was replaced singly by different carbon compounds. Care was taken that the amount of carbon in each case was similar to that present in

* It contains KNO_3 3·5 gm., $MgSO_4$ $7H_2O$ 0·75 gm., KH_2PO_4 1·75 gm., Glucose 5 gm. and Distilled Water 1000 c.c.

TABLE I

The Dry Weights in mgm. and Sporulation of Three Species of Glœosporium on Various Sources of Carbon

Glœosporium psidii					*Glœosporium limetticolum*				*Glœosporium citricolum*			
Mannitol > 103·2	Dulcitol > 84·8	Glucose > 63·9	Sorbitol > 58·1	Galactose > 55·6	Mannitol > 110·0	Raffinose > 94·4	Galactose 89·4	Sorbitol > 88·0	Mannitol > 103·9	Lactose 94·6	Raffinose 90·0	Galactose 88·7
R	R	++++	R	+++	+	++++	++++	+++	++	+++	+++	+++
Raffinose 53·6	S. Starch > 53·0	Inulin > 49·6	Maltose > 45·5	Tartaric acid 44·3	S. Starch > 78·0	Sucrose > 71·4	Malic acid 68·1	Maltose 66·0	S. Starch 87·1	Sorbitol 84·2	Dulcitol 84·0	Malic acid 77·8
++	+++	R	++	++++	++++	++++	+++++	+++++	+++	+++	+	+
Sucrose > 43·6	Rhamnose > 40·0	Lactose 36·2	Arbutin > 35·5	Dextrin > 33·9	Glucose > 64·4	Tartaric acid > 59·0	Dulcitol > 56·5	Lactose > 50·7	Dextrin 76·9	Sucrose 76·7	Tartaric acid 69·9	Glucose 60·5
++	R	+	+	++	++++	+++++	++++	++++	++	++	+	++++
Malic acid > 27·9	Arabinose > 25·7	Glycerine > 18·9	Xylose > 10·8	No. carbon 0·0	Rhamnose > 47·2	Arbutin 35·9	Dextrin 34·0	Inulin > 33·0	Rhamnose > 55·1	Maltose 45·2	Arbutin 41·8	Inulin > 40·1
+	++	R	R	–	+++	+	+	R	++	+++	++	+
					Glycerine 20·9	Arabinose > 19·6	Xylose > 4·2	No carbon 0·0	Arabinose > 29·3	Glycerine > 18·5	Xylose > 9·5	No carbon 0·0
					R	+	–	–	++	R	R	–
C.D. at 5% level = 1·17.					2·33				8·75			

5 grams of glucose (*viz.*, 2 grams of carbon). Even in media containing mixed sources of carbon the total amount from different sources was so adjusted as to include only 2 grams of carbon. The amount from each source was similar. The dry weights were determined by the usual method after the organism had grown on liquid media containing 50 c.c. of the nutrient solution in 150 c.c. pyrex flasks at 26° C. for 3 weeks. Double distilled water and purest available chemicals (Analar) were used. Fifteen different microscopic fields were examined before deciding about the degree of sporulation. The amount of sporulation has been indicated by the following sign (+).

\+ Poor
\++ Fair
\+++ V. Fair
\++++ Good
\+++++ V. Good
\++++++ Excellent

Every positive sign denotes the presence of four spores. R denotes rare sporulation (less than four).

Polysaccharides undergo some hydrolysis during autoclaving. They were, therefore, sterilized by fractional sterilization (*i.e.*, steaming them for ½ hour daily for 3 successive days).

Experimental Results

The three species of Glœosporium were grown on 19 different sources of carbon. The statistical analysis indicated that the different sources could be arranged in the order recorded in Table I.

Mannitol, galactose, raffinose and sorbitol supported good growth of the three species of Glœosporium used in the present investigation. These four compounds were, therefore, mixed together in all possible comibinations and their effect on growth and sporulation of the three fungi was studied. The results are summarized in Tables II, III and IV.

It is evident from the above tables that the behaviour of different species of the same genus may not be essentially similar towards a mixture of various compounds of carbon. The growth of the organism on the mixture could be better or worse than on individual substances.

Table I shows that mannitol supported the best growth of all the three species of Glœosporium. It has been observed that any combination of

TABLE II

Average Dry Weights in mgm. and Sporulation of G. psidii *on Combinations of Carbon Compounds*

Mixture of carbon compounds	Dry weight	Sporulation
Mannitol + raffinose	107·9	+++
Mannitol + galactose	104·0	+++
Mannitol	103·5	R
Mannitol + galactose+sorbitol+raffinose	103·5	+++++
Mannitol + sorbitol + raffinose ..	97·1	++++
Mannitol + sorbitol + galactose ..	96·2	+++
Raffinose + galactose	94·0	++++
Sorbitol + raffinose + galactose ..	92·0	+++
Mannitol + sorbitol	90·1	++
Sorbitol + raffinose	86·4	+++
Sorbitol + galactose	76·4	+++
Sorbitol	58·0	R
Galactose	55·0	+++
Raffinose	52·0	++

General mean = 86·86 ± 1·9.
C.D. at 5% level = 1·9.

TABLE III

Average Dry Weights in mgm. and Sporulation of G. limetticolum *on Combination of Carbon Compounds*

Mixture of carbon compounds	Dry weight	Sporulation
Sorbitol + raffinose + galactose ..	123·0	+++
Mannitol	111·0	+
Raffinose + galactose	105·5	+++
Sorbitol + galactose	104·6	+++
Sorbitol + raffinose	98·4	++
Raffinose	94·0	++++
Galactose	89·8	+++
Sorbitol	87·5	+++
Mannitol + galactose	87·1	++
Mannitol + raffinose	85·7	+++
Mannitol + sorbitol+galactose+raffinose	82·4	+++
Mannitol + sorbitol + galactose ..	80·0	++
Mannitol + sorbitol + raffinose ..	75·4	++
Mannitol + sorbitol	62·4	+

General mean = 91·78 ± 4.833
C.D. at 5% level = 4·833.

TABLE IV

Average Dry Weights in mgm. and Sporulation of G. citricolum *on Various Combinations of Carbon Compounds*

Mixture of carbon compounds	Dry weight	Sporulation
Mannitol	104·5	++
Mannitol + galactose	99·3	+++++
Mannitol + galactose + sorbitol ..	99·1	++++
Raffinose	89·4	+++
Galactose	88·0	+++
Mannitol + sorbitol	86·6	++++
Mannitol + raffinose	85·1	++++
Sorbitol	83·8	+++
Mannitol + raffinose + sorbitol + galactose	77·6	++++
Mannitol + sorbitol + raffinose ..	75·2	++++
Raffinose + galactose	69·5	++++++
Sorbitol + galactose	65·0	++++++
Sorbitol + raffinose + galactose ..	57·0	++++
Sorbitol + raffinose	44·0	++++

General mean = 80·29 ± 5·45.

C.D. at 5% level = 5·45.

mannitol with galactose, raffinose or sorbitol significantly decreased the growth of *G. limetticolum* and *G. citricolum* but *G. psidii* could utilize it with significantly greater advantage when it was mixed with raffinose. There was no significant difference between mannitol and a combination of this substance with galactose or with a mixture of all the four substances. In all other combinations the growth was significantly less.

Individually galactose, raffinose and sorbitol were the poorest source of carbon for *G. psidii* but the growth was significantly better on any of their combinations. Similarly their mixtures were better for *G. limetticolum.* A combination of all the three substances gave best growth of this organism. The growth of *G. citricolum* on different combinations of raffinose, galactose and sorbitol was generally less than on their individual sources. Their presence even decreased the growth on mannitol.

There was a marked influence of the mixture of carbon compounds on sporulation. Excellent sporulation of *G. psidii* was observed on a mixture containing all the four substances. *G. citricolum* also behaved similarly as the sporulation was better on various mixtures. Excellent sporulation was observed on a mixture of raffinose and galactose as well as on sorbitol

and galactose. It was very good on a combination of mannitol and galactose. Other combinations gave good sporulation though none of the four compounds could support good sporulation when they were added singly to the medium.

In general the sporulation of *G. limetticolum* was poorer and it was less on the mixture of different compounds than on individual substances. The best sporulation was on raffinose. The effect of different combinations of carbon compounds was particularly marked on sporulation.

DISCUSSION

All the three species of Glœosporium gave very good growth on mannitol but it supported poor sporulation. Hawker (1939) also found that mannitol was a poor source for the sporulation of *Melanospora destruens*. The higher alcohol (mannitol) if mixed with raffinose, galactose or sorbitol improved the sporulation of *Glœosporium psidii* and *Glœosporium citricolum*. The sporulation of *Glœosporium limetticolum*, however, was suppressed when mannitol was combined with either of the above three substances. This ability or inability to utilize certain combinations of carbon compound for reproduction can be best explained as an inherent quality of an organism.

Mosher *et al.* (1936), Corum (1942), Matsumato (1921) and Ledeboer (1934) reported that galactose was a good source for the growth of *Trichophyton interdigitale*, *Rhizopus suinus*, Rhizoctonia Species and *Ceratostomella ulmi* respectively. Horr (1936) and Edgecombe (1938), however, found that it decreased growth and reduced sporulation of *Aspergillus niger*, and Penicillium Species, etc., when it was used as the sole source of carbon. The three species of Glœosporium under investigation, however, developed significantly good growth on galactose. Lilly and Barnett (1953) also reported that galactose was a good source for *Colletotrichum phomoides* and *Colletotrichum lindemuthianum*. Horr (1936) as well as Hawker (1939) mentioned that *A. niger* and *Melanospora destruens* utilized galactose better when it was mixed with some additional source of carbon. In the present investigation also the growth of *G. psidii* improved when galactose was mixed with mannitol, raffinose or sorbitol but every combination of those substances failed to improve the growth than any of them alone while galactose and mannitol increased the growth of *G. citricolum*.

Raffinose is found in many plants. Lilly and Barnett (1953) have mentioned that *Fusarium medicaginis*, *Phoma betæ* and *Sphæropsis malorum* could grow well on this substance. Hawker (1936), however, found it to be an inferior source of carbon. According to her those results were obtained

because of the general unsuitability of galactose which is one of the products of hydrolysis of this sugar. In the present investigation galactose is found to be a good source of carbon and the hydrolysis of raffinose is thus not likely to have any adverse effect. Leonian (1925) found that raffinose was more favourable for the production of sporangia of *Phytophthora* sp. This substance could increase both the growth and sporulation of *G. psidii* and its combination with others improved the sporulation of *G. citricolum*. A combination of raffinose and mannitol decreased the growth of *G. limetticolum* though it was better for *G. citricolum*.

Wolf *et al.* (1950) found sorbitol to be useless for *Monosporium apiospermum* but Grewal (1954) reported it to be a good source of carbon for the sporulation of *Glœosporium papayæ* and *Colletotrichum papayæ*. All the three fungi grew well on sorbitol. It has already been mentioned that *G. psidii* grew better on a mixture of several carbon substances than on sorbitol alone which increased the growth of *G. limetticolum* in any combination of raffinose and galactose but *G. citricolum* gave improved growth in combination with mannitol alone and not with raffinose and galactose. This clearly indicates that addition of mannitol to any of the three substances improved growth of *G. psidii* and *G. citricolum* even though its addition suppressed the growth of *G. limetticolum*. According to Lilly and Barnett (1953) such organisms are able to produce small quantities of several enzymes more easily than a large quantity of anyone alone. Such fungi which like mixed carbon sources are probably better fitted for life under natural conditions where mixtures are frequent than larger quantities of any single form. Their capacity to sporulate better on mixtures of various sources of carbon may help them to survive in nature.

Summary

1. Three species of Glœosporium were isolated from guava, lime and citron twigs. Their behaviour towards the mixture of various compounds of carbon were not similar.

2. *G. psidii* and *G. limetticolum* grew significantly better on any combination of galactose, raffinose and sorbitol while *G. citricolum* supported less growth than on their individual sources. Mannitol alone supported best growth of *G. citricolum* but it could produce excellent growth of *G. psidii* when it was mixed with raffinose.

3. Excellent sporulation of *G. psidii* was observed on a combination of all the four carbon substances, that of *G. citricolum* on a mixture of raffinose and galactose or sorbitol and galactose. *G. limetticolum* gave best sporulation on raffinose alone.

REFERENCES

1. Corum, C. J. .. "Carbohydrate nutrition of *Rhizopus suinus*," *Bot. Gaz.*, 1942, **103**, 740–50.
2. Edgecombe, A. E. .. "The effect of galactose on the growth of certain fungi," *Mycologia*, 1938, **30**, 601–24.
3. Grewal, J. S. .. *D.Phil. Thesis*, University of Allahabad, 1954.
4. Hawker, L. E. .. "The influence of various sources of carbon on the formation of perithecia by *M. destruens* in the presence of accessory growth factors," *Ann. Bot. N.S.*, 1939, **3**, 455–68.
5. ———— .. *Physiology of Fungi*, University of London Press, Ltd., London, 1950.
6. Horr, W. H. .. "Utilization of galactose by *A. niger* and *P. glaucum*," *Plant Physiol.*, 1936, **11**, 81–99.
7. Ledeboer, M. S. J.* .. "*Physiologiche onderzœkinjen over Ceratostomella ulmi*," *Buisman Dissert, Utrecht*, 1934.
8. Leonian, L. H. .. "Physiological studies on the genus *Phytophthora*," *Amer. Jour. Bot.*, 1925, **12**, 444–98.
9. Lilly, V. G. and Barnett, H. L. "Utilization of sugars by fungi," *West Virginia University Tech. Bul.*, 1953, 362.
10. Matsumato, T.* .. "Morphology and Physiology of fifteen isolates of Rhizoctonia," *Ann. Mo. Bot. Gard.*, 1921, **8**, 1–62.
11. Mosher, W. A., Saunders, D. H., Kingery, L. B. and Williams, R. J. "Nutritional requirements of the pathogenic mold *T. interdigitale*," *Plant Physiol.*, 1936, **11**, 795–806.
12. Steinberg, R. A. .. "Growth of fungi in synthetic nutrient solutions," *Bot. Rev.*, 1939, **5**, 327–50.
13. Tandon, R. N. and Agarwala, R. K. "Pathological studies of *Gloeosporium psidii* causing Die-back of guavas," *Proc. Ind. Acad. Sci*, 1954, **40**, 102–09.
14. Wolf, F. T., Bryden, R. R. and Maclaren, J. P. "The nutrition of *Monosporium apiospermum*," *Mycologia*, 1950, **42**, 233–41.

* Not referred to in the original.

STIPULES, STIPELS, LIGULES AND LEAF-SHEATH*

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Introduction

Stipule, stipel, ligule and leaf-sheath are structures closely associated with the foliage leaf. The first three are regarded as outgrowths of the leaf-base, and the last as " a primary element " of the leaf (Arber, 1925; Majumdar, 1955). The *stipule*† is defined as " an appendage at junction of a leaf, one on each side of the insertion " (Asa Gray, 1879); " an outgrowth of the base of the leaf " (Willis, 1951). The *stipel* when present is described as " the stipule of a leaflet usually one at the base of each in a compound leaf with two at the base of the terminal leaflet " (A, B). The *ligule* is " an outgrowth from the leaf-sheath or petiole of the Grasses where it joins the blade " (A); " a scale at the upper end of the leafsheath " (B). The *leaf-sheath* is " a close fitting tubular or enrolled case as that formed by the stem-clasping petioles (*i.e.*, lower portion of the leaf) " (A); " leaf-base forming a tube round the stem " in Grasses (B).

Thus the stipules may be an adjunct or an appendage of a leaf (A), or an outgrowth of the base (leaf-base)‡ of the leaf (B); stipels are secondary stipules associated with leaflets of a compound leaf (A, B); the ligule is an outgrowth of the leaf-sheath (A) or of the sheathing base of the leaf (B), and the leaf-sheath is the dilated base of the leaf or the petiole (A), or the sheathing leaf-base (B).

We propose to examine them individually with a view to determine their true morphology on the results of modern researches including those of our own.

---

* The following are the outstanding contributors on stipules, ligules and leaf-sheath: Clos (1879), Colomb (1887), Tyler (1897), Lubbock (1899), Glück (1901), Goebel (1905), Domin (1911), Sinnott and Bailey (1914), Arber (1925), Philipson (1935), Parkin (1948), Mitra (1945, 1948, 1949 *a-b*, 1950 *a-c*).

† Abbreviations used in this paper: A and B represent American and British botanists respectively. Median and lateral for the median and lateral bundles of the leaf-trace. Two definitions, one American and one British, of each structure are given.

‡ *Leaf-base* and *base of the leaf* should be distinguished: Leaf-base is 'a primary element of the leaf' (B), whereas the base of the leat is the lower portion of the leaf, *i.e.*, the base of the petiole (A).

THEIR MORPHOLOGICAL STATUS

Stipule.— American botanists regard stipules as one of the three parts of a complete leaf; they do not recognize the presence of the leaf-base as a separate entity. The German and British botanists on the other hand, regard the leaf-base as an integral part of a complete leaf, and the stipules when present they regard as outgrowths of the leaf-base (Majumdar, 1955 *b*). It is quite natural, therefore, for Eames and MacDaniel (1925) to state that " the morphological nature of stipules has been much in dispute ".

Researches of Colomb, Sinnott and Bailey, Dormer (1944), Mitra and Sharma (1955) have now definitely settled the question of the morphology of this organ. *It is an outgrowth of the leaf-base caused by the stimulating influence of a branch or branches sent out by the laterals* while on their way to the median with which they unite to form the petiolar trace. Mitra has further shown that whatever be the type of the stipule—free lateral, adnate, interpetiolar, intrapetiolar, foliaceous, bud-scale or ocreate*—the development of each one of them is correlated with the lateral trace bundles of the leaf with which they are associated. In the majority of cases, the trace of a stipulate leaf consists of three bundles—the median and two laterals, but in some special plants it may consist of many bundles (node-multilacunar).

Stipel.—They are secondary stipules associated with the leaflets of a compound leaf. We have already mentioned that the lateral leaflets have one stipel each, and the terminal one has a pair. Kar (1955) has worked out the origin and vascularization of the stipels of *Dolichos lablab* L. He has shown that the stipel of a lateral leaflet receives branches from the same lateral which goes to form the vascular system of the leaflet with which it is related. *This supports their stipular nature.*

The presence of a single stipel to each lateral leaflet and a pair to the terminal one has also been explained by Kar on the evidence of their vascularization (see Kar, 1955).

Ligule.—Ligules like stipules are outgrowths of the leaf-base. Stipulate leaves are more common in dicotyledons and ligulate leaves in monocotyledons, particularly, in the Grasses and the Zingiberaceæ. They are generally associated with the sheathing base of the leaf arising " from the junction of the leaf-blade and leaf-sheath ". Under ligule we shall consider here only the ligule of Grasses.

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* Stipular tendrils of Smilax has not been considered here. Chakravarti and Mitra (1948) have shown that these tendrils "originate, as pointed out by Arber in 1920, by chorisis or splitting of the petiole after it has separated from the sheathing base during the development and differentiation of the leaf primordium" (p. 27).

We have stated under the Section Stipule that the stipular traces are always derived from the branches of the laterals of a three and sometimes many-bundled leaf trace. Colomb applied this dictum to the ligules of *Arundinaria japonica* and *Oryza sativa* and found that the ligules of these plants are dual in structural organization: its two margins are prolongations of the margins of the leaf-sheath and the central portion is the " upgrowth of the adaxial (ventral) surface of the sheath joining these, *i.e.*, stipular " (Philipson, 1935) as it receives only the branches of the laterals. Philipson and Saha (1952) supported Colomb while interpreting respectively the ligules of *Phyllostachys aurea* and of Rice plant. *The ligule of Grasses therefore is a composite structure made up of the margins of the leaf-sheath and its stipule.*

Leaf-sheath.—The majority of American botanists consider the leaf-sheath to be the product of the base of the leaf or the petiole which becomes " much dilated and membraneous at base " (Asa Gray, p. 104). But Sinnott and Bailey (1914) think that the leaf-sheath is formed by the fusion of rows of adjacent stipules (one opposite each lateral in a multilacunar node) (p. 448).

The European botanists on the other hand regard the leaf-sheaths as modified leaf-bases as in the Umbellifers and Monocotyledons. Curiously enough Parkin (1948) supports the stipular origin of the leaf-sheath (p. 70).

I have discussed the morphological nature of the leaf-sheath in my paper on " The Complete Foliage Leaf " (1955 *b*). In that paper I have shown that even when the lamina and the petiole are absent, either simultaneously or one at a time, the leaf-base, as a primary element, is always present. A leaf without a base cannot thus be conceived.

The leaf-base may remain included entirely in the axis as its outer component or the mantle, or a part of it may develop free from the axis as the *leaf-sheath*. The development of the leaf-sheath is closely associated with the trilacunar, as in Rose, but mainly with the multilacunar conditions of the node, as in Heracleum, Polygonum and Centella.

Whether the leaf-base will develop into a free base or not is determined by the behaviour of the laterals during their courses through it towards the base of the petiole. The laterals after their departure from the axial cylinder run parallel for a short or long distance through the axial component and then gradually or abruptly shift towards the median. The laterals and the median come together and unite at the base of the petiole and then enter the same as its trace. In the petiole they do not ordinarily branch nor separate from one another (pp. 68–69; *cf.* Sinnott and Bailey, 1915; Swamy, 1949).

During its ontogeny the leaf primordium starts as *soubassements foliares* (Gregoire, 1935; Louis, 1935) with one provascular strand, the median. It then extends laterally around the free apex when more bundles come from the axial cylinder in twos, one on each side of the median. The node becomes tri- or multi-lacunar depending on the extent of the wings around the apical dome (for details see Majumdar, 1955 *b*).

Modern researches, therefore, conclusively prove the existence of the leaf-base even where it is apparently absent, as in China Rose and *Morus alba*. The *leaf-sheath* is the extended portion of the leaf-base free from the axis, formation of which is determined by the behaviour of the laterals in it (Majumdar, 1955 *b*).

PHYLOGENETIC CONSIDERATION AND CONCLUSIONS

Only the stipule, ligule and the leaf-sheath will be considered here. The question is: Are these structures independent morphological entities or they are derived one from the other and phylogenetically connected ? Verdicts of four plant morphologists are examined below. They are Sinnott and Bailey, Arber and Parkin: the former two are American and the latter two are British eminent and foremost botanists. *They are all living.*

Sinnott (1914) worked on the anatomy of the node as an aid in the classification of angiosperms. He studied and analyzed 400 genera and came to the conclusion that the trilacunar condition of the node is the primitive form from which the unilacunar condition has been derived either by the suppression of the laterals or by their approximation and juxtaposition with the median, and the multilacunar condition, by the amplification, *i.e.*, by an increase in the number of laterals of the trace of a leaf.

In the same year Sinnott and Bailey in their paper on the nodal anatomy and the morphology of the stipules came to the following conclusions:

(i) Stipules are associated with the trilacunar condition of the nodes; unilacunar nodes normally bear exstipulate leaves, and sheathing bases are connected with the multilacunar condition;

(ii) in the formation of the stipules branches are sent out from the lateral trace bundles which stimulate their origin and growth and form their vascular system. In this conclusion they supported the findings of Colomb (1887); and

(iii) the leaf-sheath is formed by the fusion of rows of adjacent stipules on the two sides of the petiole, each stipule of a row is being stimulated to growth by a branch of the lateral of the multibundle leaf trace.

Arber (1925, refer to her Figs. 74 A–H, p. 97) discussed the leaf-sheath, stipule and ligule somewhat in detail in her classical work, Monocotyledons. She considers the simple leaf-sheath as the fundamental type (A) " from which stipules and ligules are developed by a secondary process of winging " (p. 97). Then she describes the steps in their evolutions: " By a simple upward extension of the leaf-sheath, where it abuts on the petiole a ligulate type (B) is easily derivable from A. The upper lateral margins of the leaf-sheath may on the other hand grow out into two wings (C), which by reduction of the basal part of the sheath may come to form stipules of the free lateral type (D). As a further modification the wings of the leaf-sheath may fuse with greater or less completeness in front of the petiole base (E, H), and from such types an ' axillary ' stipule or ligule (G, H) may be developed by reduction of the rest of the leaf base. H may, alternately, be derived by the same process from such a form as B". "In the case where the position is axillary stipules and ligules are synonymous, the former distinguished from the latter by the bifurcation at the tip (G, H), the bifurcation indicating the double nature of the pair of stipules." According to Arber the ligule of Grasses is an axillary stipule.

Parkin (1948) has considered the stipule phylogenetically. He divided different kinds of stipules into two broad categories: Cauline (free) and petiolar (adnate) (p. 65). He concluded that " a pair of free stipules is an original accompaniment of the angiospermous leaf " (p. 79). This conception is based on Sinnott and Bailey's (1915) conclusion that the primitive angiospermous leaf was trilobed from which Parkin speculated that the two lateral lobes became " modified as stipules for protective purposes and that the middle lobe became transformed into the simple oval leaf (1953, p. 85). He thus regarded the free cauline stipules as earlier forms from which all other kinds of stipular outgrowths could be theoretically derived; adnate stipules are the result of the fusion of the pair of free lateral (cauline) stipules with the petiole, and he believed that the sheath and the ligule are of stipular origin rather than as peculiar outgrowths of the leaf base. He doubted " the leaf-base as a morphological element of the foliage on a par with lamina and petiole" (p. 80). In this respect he thus holds the same view with Sinnott and Bailey. Finally as a corollary of his above view he believed that the leaf-sheath had been derived from the adnate stipules.

These conflicting views held by eminent plant morphologists on the true morphology and phylogeny of the stipule, ligule and leaf-sheath appear to me to be due to the want of a clear and proper realization of the presence, nature and extent of the leaf-base as a morphological element of the foliage leaf.

In 1851 Hofmeister propounded his Berindung Theory which postulated the existence of an outer mantle formed from the leaf-bases enveloping a core in the make-up of the axis (in Saunders, p. 145). Nobody apparently took notice of this theory. In 1922 Saunders revived this theory in a modified form and postulated her Leaf-Skin Theory. She firmly established the fact that the outer skin of the axis (internode) is made up of the bases of the leaves (leaf-bases). Arber (1925) considered this theory of Saunders somewhat in detail and without any commitment she wrote: " if we accept the Leaf-Skin Theory we cannot escape the conclusion that the leaf plays a much more important rôle in the organization of the shoot than has hitherto been generally held " (p. 93). She compares the leaf of the text-book with the visible part of an iceberg and the leaf-base forming the outer layers of the axis, to the portion below the sea-level. In 1952 Mitra and Majumdar re-examined both the theories of Hofmeister and Saunders in the light of modern researches and established the correctness of the Leaf-Skin Theory but supported Hofmeister in so far as the radial depth of the leaf-skin is concerned. Sharman (1942) has also shown that in maize stem each internode is made up of the lower half of the leaf cushion. *The existence of the leaf-base as an element of the leaf can therefore no longer be doubted.*

I now propose to examine the view-points of Sinnott and Bailey and Arber and Parkin on the morphology and phylogeny of the stipule, ligule and leaf-sheath on the basis that the leaf-base exists as a ' primary element ' of the foliage leaf. I even go so far as to state that even where the lamina and/or the petiole is absent its base is present as the outer component of the axis (leaf-skin) and as the bud-scale in some cases (Majumdar, 1955 *b*).

We have already indicated before that in the angiospermous leaves the leaf-base exists in two forms: In the majority of cases where the petiole appears directly seated on the axis it exists fully incorporated into the axis as its outer mantle (skin), so that to the uninitiated the leaf appears without a leaf-base. This led the American botanists to deny the existence of the leaf-base in foliage leaves, and as a corollary they consider the leaf-sheath as the dilated base of the leaf or the petiole. Accepting this position Parkin described the stipules as arising directly from the axis (his cauline stipules) and Cross (1937) described the stipules of *Morus alba* as originating from the leaf-stem transition region (see Majumdar and Mitra, 1948).

Hofmeister's Berindung and Saunders' Leaf-Skin Theories apparently had no influence on the attitude of these botanists. Curiously enough Sinnott and Bailey (1914) who established firmly the fact of the correlation of the stipule development with the laterals of the leaf trace with a conclusion that

the " lateral trace exerts a stimulating influence which results in the formation of the stipule " are silent as to the morphology of the region of which they are the outgrowths.

The other form in which the leaf-base exists is a more common form among monocotyledons and in a few herbaceous dicotyledons. It exists partly incorporated into the axis and partly as the free leaf-sheath, *e.g.*, in Heracleum, Polygonum, Centella and Rose.

In all cases, without any exception, the stipules are leaf-base outgrowths correlated with the laterals of the trace of the leaf with which they are associated (Mitra, 1945–50).

In the so-called adnate stipules of Roses, the central radially thickened portion is regarded as the petiole and the two wings as the pair of stipules adnate to it. In 1949 Mitra published his paper "On the Origin, Development and Morphology of Adnate Stipules in *Rosa centifolia* L.". On morphogenetic and anatomical evidence he showed that the ' sub-petiolar ' region of the adult leaf of Roses is the sheathing leaf-base, only the two distal free lobes of this sheath at the sides of the petiole and whose traces are derived from the branches of the laterals represent the pair of free stipules. Thus he supported the morphological interpretation of the stipules of Roses given by Gœbel (1908) and Vines (1910) (for details see Mitra, 1949).

Arber (1925) suggested that " there is no hard and fast line between the anatomical characters of petioles and leaf-sheaths " (p. 112). We have seen above that modern researches do not support this statement of Arber. Mitra and Majumdar (1952) showed that anatomically the three regions of a typical leaf, namely, the base, the petiole and the upper leaf, could be distinguished. In the *base* the laterals remain separate, laterally spread and unbranched or branched in connection with stipule formation; in the *petiole* they unite with the median to form a cylinder or an arc or branch and arrange themselves in a ring without branching and in the *upper leaf* they again branch in connection with leaflet or lamina development (p. 365; *cf.* Sinnott and Bailey, 1915; Swamy, 1949 and Majumdar, 1955 *b*). Subsequent investigations have supported the correctness of the above observations of Mitra and Majumdar.

The ligule of Grasses is a stipule *plus* the vertically extended margins of the sheathing leaf-base. We have already discussed its morphology. Arber (1925) from its bi-fid nature regarded ligule as an axillary stipule, but Saha's (1952) developmental studies of the ligule of Rice plant have shown that the bi-fid nature of the ligule is the result of differential growth of the margins of the sheathing base and of the stipule.

Morphogenetic studies at the growing apex show that the leaf-sheath is the upward extension of the leaf-base free from the axis. Mitra and Majumdar (1952) have shown that the number and behaviour of the laterals through the axial component (leaf-base) determine the nature of the leaf-base. Only when the laterals run parallel to one another beyond the upper end of the axial component before they bend towards the median the base has a free growth, as in Heracleum, Polygonum and Centella and the sheathing base of the monocotyledons. Therefore our studies do not support Sinnott and Bailey and Parkin when they state that the sheath is formed by the fusion of the rows of adjacent stipules. Stipules themselves are leaf-base outgrowths, and ocrea and ligule are stipules outgrown from the sheathing bases of Polygonum and Grasses and Zingibers. Therefore to consider the leaf-sheath as formed from the stipules is like putting the cart before the horse.

Arber's idea that the sheathing base is the primitive type from which stipules and ligules have been derived may be partially true so far as the ocrea and ligules are concerned as they are the outgrowths of the sheathing base. But to derive the so-called cauline stipules from the sheathing leaf-base is rather difficult to accept with our present state of knowledge of the ontogeny of these structures.

If ontogeny of an organ is the recapitulation of its phylogeny then the unilacunar condition of the node should be regarded as the earliest from which tri- and multi-lacunar conditions came to be evolved. If that is so the sheathing base should come last in the phylogeny of the leaf-base. This goes counter to the findings of Sinnott (1914).

Sinnott and Bailey (1915) from their elaborate and exhaustive studies of vascular plants came to the conclusion that the primitive angiospermous leaf was palmately lobed with three major veins, one to each lobe. From this Parkin speculated that the two basal lobes of this primitive leaf became modified into a pair of free lateral stipules (see also Arber, 1950, regarding origin of axillary bud; Majumdar, 1955 *a*).

We have stated before that stipules and ligules are inseparably associated with the branching and branches of, and not directly with, the laterals of the leaf trace. The two lateral lobes of the primitive angiospermous leaf received lateral bundles and not their branches. Therefore they can be modified for any purpose other than to give origin to the pair of stipules as suggested by Parkin.

Stipules, stipels, ligules and the leaf-sheaths are primarily protective in their function. It is possible, therefore, that their origin took place in response to that function.

In the early stage of its origin the angiospermous leaf consisted of the eaf-base and the lamina, the former entirely incorporated into the axis as its outer mantle, and the latter with a broad base seated directly on the axis. The node was perhaps unilacunar. Thus the broad sessile base of the lamina, at this stage, gave all the necessary protection to its axillary bud. There was no petiole at this initial stage of leaf origin.

According to Sinnott and Bailey (1915) " the ever-increasing broad base of the lamina was in need of a greater flexibility " for proper exposure to sunlight, against wind, etc. Therefore a petiole was next added between the base and the lamina. The lateral bundles (two or many) which were more or less widely separated from each other at their departure from the axial cylinder were forced to come closer in the petiole. Morphogenetic studies at the shoot apex also show that the petiole is the last part to appear in the ontogeny of a leaf primordium, and is " more recent in evolutionary ontogeny than the other two parts of the leaf" (Sinnott and Bailey, 1915; Majumdar, 1955 *b*).

The broad base of the lamina was now separated from the leaf-base (axial component) by the petiole whose base was too narrow to give adequate protection to the axillary or the terminal bud. The demand for additional protection was met by the development of a pair of stipules from the leaf-base on the two sides of the petiole. The three together formed an effective protective structure. There are many instances where the petiole development is soon arrested and the pair of stipules grow much quicker and bigger to cover not only the axillary bud but the growing point as well. The condition of the node was perhaps trilacunar in most of the cases.

The origin of the stipule may thus be taken as a consequence of the addition of the petiole to the leaf.

Meanwhile the *soubassements foliares* started extending around the apical dome until it completely embraced the same. With this extension more bundles from the axial cylinder entered the wings of the axial component as its laterals and the node became multilacunar.

When the node became multilacunar, the leaf alternate and the axial component completely surrounded the axis the petiole with the two small stipules at its base was not enough to ensure protection to the axillary and terminal buds. This vulnerable situation was met by the leaf-base (axial component) in two ways:

In plants like Magnolia, Michelia, *Ficus elastica*, *F. religiosa* and *Artocarpus integrifolia* the leaf-base remained as axial component but produced stipules which either singly or in pairs completely ensheathed the apical or

axillary bud as bud-scales, the petiole seldom taking any part in the protective function. The vascular supply as usual came from the branches of the laterals.

In Monocotyledons, such as the Grasses and the Zingiberaceæ, and in some herbaceous dicotyledons, such as the Umbellifers and Polygonums, where the internodes become very long during the unfolding of the buds, the laterals do not change their courses at top of the axial component but pursue their vertical course beyond the latter thereby causing its upward extension free from the axis *to form the leaf-sheath.*

The leaf-sheath itself affords protection to the axillary bud but it does not as adequately protect the vegetative bud and its delicate growing tip particularly when the environment is inclement. In such cases an additional stipule, ocrea or a ligule, is formed.

Summary of the stages through which the leaf-base is supposed to have passed during its phylogeny

Forms of the leaf-base	Nodal condition	Leaf : sessile or petiolate	Exstipulate, stipulate or sheathing leaf-base	Protection : how effected ?
A Leaf-base entirely incorporated into the axis as axial component; no free leaf-base	1 Unilacunar or trilacunar ?	Sessile	Exstipulate	*By the broad base of the lamina
	2 Trilacunar	Petiolate	Stipulate	By the petiole *plus* the pair of stipules
	3 Multilacunar	Petiolate	Stipules forming sheathing bud-scales	By the stipules forming a complete sheath covering the bud
B. Leaf-base partly incorporated into the axis and partly as the free leaf-sheath	1 Trilacunar	Petiolate	Leaf-sheath	Sheathing base, *e.g.*, Roses
	2 Multilacunar	Petiolate	Exstipulate	By sheathing leaf-base : leaf-sheath, *e.g.*, Centella
	3 Multilacunar	Petiolate	Sheathing	Leaf-sheath *plus* ocrea or ligule

* Primitive leaf.

We may now conclude: The primitive angiospermous leaf consisted of the *leaf-base* wholly incorporated in the axis as the axial component, and the *lamina* with a broad base directly inserted on the axis. The node was perhaps unilacunar. With the addition of the *petiole* to the leaf *a pair of stipules* came into being (cauline of Parkin). The node was trilacunar at this stage. The axial component meanwhile extended laterally around the apical dome and finally completely enclosed the latter and the node became multilacunar. It then produced the *sheathing stipules* of the type of bud-scales. In the next

stage the axial component extended vertically under the influence of its own laterals to produce the *leaf-sheath*. But this was found to be inadequate to afford complete protection to the apical bud in certain types of plants. Therefore stipules of the types *ocrea* and *ligules* were added.

The stipules, stipels and ligules are homologous structures, for without exception all of them are outgrowths of the leaf-base correlated with the branches of the laterals. The leaf-sheath as extension of the axial component developed later as it is usually found in herbaceous plants.

The phylogenetic history of development of the leaf-base through ages has been written above on two basic inferences, *viz.*, (1) that there exists the leaf-base as " a primary element " of *all* leaves, and (2) that the primary functions of the leaf-base and its outgrowths are protective and to some extent mechanical.

Summary

1. Stipules, stipels, ligules and leaf-sheaths have been discussed ontogenetically and phylogenetically with a view to examine their true morphology.

2. In this connection conflicting verdicts of Sinnott and Bailey and Arber and Parkin have been critically examined. It is felt that in arriving at their conclusions they failed to take into their account the presence, nature and extent of the *leaf-base* as a morphological unit constituting the foliage leaf.

3. Modern researches have proved beyond doubt that the leaf-base exists as " a primary element " of leaves of every kind.

4. The leaf-base exists in two forms: (1) totally incorporated into the axis as its outer mantle (Hofmeister, Saunders) described in this paper as the axial component, and (2) partly as the axial component and partly as the leaf-sheath which is the vertical extension of the former free from the axis.

5. Stipules, stipels and ligules are without exception outgrowths of the leaf-base formed only under the influence of branches of the laterals of the leaf trace.

6. Where the leaf-base exists only as the axial component the leaf is apparently without a base, and the casual observer is led to believe in such cases the origin of stipules as cauline (*cf.* Parkin).

7. As stipules are outgrowths of the leaf-base they cannot by their fusion give rise to the leaf-sheath (*cf.* Sinnott and Bailey, Parkin).

8. In Roses the subpetiolar region is the leaf-sheath (leaf-base) and its two distal free lobes on the two sides of the petiole represent the stipules (adnate of Parkin and others).

9. In ocrea only the upper portion fed by the branches of the laterals is to be regarded as the stipule and the lower portion in which the laterals run separate, almost parallel, courses is the leaf-sheath (leaf-base).

10. Ligules of Grasses are composite structures (stipule *plus* margins of the leaf-sheath) (Colomb, Philipson, Saha).

11. The leaf-base, petiole and the lamina of a leaf can be distinguished, one from the other on anatomical evidence (*cf.* Arber).

12. Derivation of the stipules and ligules from the leaf-sheath as suggested by Arber, is not supported.

13. If ontogeny of an organ is the recapitulation of its phylogeny then the unilacunar condition of the node is the primitive from which the trilacunar and then the multilacunar conditions must have evolved.

14. There is no direct evidence from which it can be definitely concluded that the two basal lobes of the primitive angiospermous leaves (Sinnott and Bailey) with three main bundles became modified into the pair of stipules at the base of the petiole (*cf.* Parkin).

15. Sheathing leaf-base (leaf-sheath) is the result of multilacunar condition of the node *plus* the behaviour of the laterals in the axial component. Leaf-sheath and sheathing stipules are morphologically different and distinct structures.

16. Stipules, ligules and leaf-sheaths are structures primarily meant for affording adequate protection to the axillary and terminal buds and their constituent parts. It is probable that the necessity of a pair of stipules, free or sheathing, and the leaf-sheath with ocrea and ligule arose after the addition of the petiole to the leaf.

17. Stipules, stipels and ligules are homologous structures. A tentative scheme of phylogeny of the stipules, ligules and leaf-sheaths has been suggested.

LITERATURE CITED

Arber, A. .. *Monocotyledons*, Cambridge, 1925.

——— .. *The Natural Philosophy of Plant Forms*, Cambridge, 1950.

Asa Gray .. *Structural Botany*, 1879, **1**. N.Y.

Chakravarti, H. L. and Mitra, G. C. "Developmental Studies: Tendrils of *Smilax microphyla*," *Bull. Bot. Soc. Beng.*, 1948, **2**, 22.

* Clos, D. .. "Independence, Development, Anomalies des Stipules, etc.," *Bull. Soc. Bot. Fr.*, 1879, **26**, 189.

Colomb, G. .. "Recherches sur les Stipules," *Ann. Sci. Nat.*, 1887, **7** (6), 1.

Cross, G. L. .. "The origin and development of foliage leaves and stipules of *Morus alba*," *Bull. Torr. Bot. Club*, 1937, **24**, 266.

*Domin, K. .. "Morphologische und Phylogenetische Studien uber die Stipularbildungen," *Ann. Jard. Bot. Buitenzorg.*, 1911, **2** (9), 117.

Dormer, K. J. .. "Some examples of correlation between stipules and lateral leaf traces," *New Phyt.*, 1944, **43**, 151.

Eames, A. J. and MacDaniels, L. H. *An Introduction to Plant Anatomy, N.Y.*, 1925.

* Gluck, H. .. "Stipulargebilde der Monokotyledonen," *Verhandl. d. Naturhist.-Med. Ver zu Heidelburg. N.F.*, 1901, **7**, heft 1, 1.

Goebel, K. .. *Organography of Plants*, Eng. ed., Part II, Oxford, 1905.

Gregoire, V. .. "Donees nouvel les sur la morphogenese de l'axe feuille dans les Dicotylees," *C.R. Acad. Sci. Paris*, 1935, 200.

* Hofmeister, W. .. "Vergv. Untersuchungen, etc." (in Saunders, 1922), 1851.

Kar, A. .. "Stipules and stipels of *Dolichos lablab* L. and their vascular supply," *Proc. Nat. Inst. Sci.*, 1955, **21 B**, 137.

Lawrence, G. H. M. .. *Taxonomy of Vascular Plants, N.Y.*, 1951 (A).

Louis, J. .. "L'Ontogenese du Systeme Conducteur dans la pousse feauille dans Dicotylees et des Gymnospermes," *La Cellule*, 1935, **44**, 87.

Lubbock, J. .. *On Buds and Stipules*, London, 1899.

Majumdar, G. P. .. "The Foliage Leaf and the Axillary Bud," *Trans. Bose Res. Inst.*, 1955 *a*, **22**, 87.

———— .. "The Complete Foliage Leaf," *Proc. Ind. Acad. Sci.*, 1955 *b*, **42 B**, 65.

——— and Mitra, G. C. .. "The origin and development of Stipules in *Morus alba* L.," *Bull. Bot. Soc. Beng.*, 1948, **2**, 1.

Mitra, G. C. .. "The origin, development and morphology of the ocrea in *Polygonum orientale*," *J. Ind. Bot. Soc.*, 1945, **26**, 191.

———— .. "Developmental Studies: The interpetiolar stipules of Rubiaceæ with special reference to *Pæderia fœtida* and *Ixora parviflora*," *ibid.*, 1948, **27**, 150.

———— .. "The origin, development and morphology of adnate stipules in *Rosa centifolia*," *ibid.*, 1949 *a*, **28**, 68.

———— .. "Comparative account of the development of the base of the sheathing, the stipulate and exstipulate leaves of four species of dicotyledons," *Bull. Bot. Soc. Beng.*, 1949 *b*, **3**, 33.

———— .. "Developmental Studies: The origin, development and morphology of the foliaceous stipules of *Pisum sativum*," *Proc. Ind. Acod. Sci.*, 1950 *a*, **31 B**, 40.

———— .. "Origin, development and morphology of the intrapetiolar stipules of *Ervatamia divaricata*," *Bot. Gaz.*, 1950 *b*, **112**, 106.

———— .. "Developmental Studies: A comparative account of the origin, development and morphology of the stipules of *Artocarpus integrifolia, Ficus religiosa* and *F. elastica*," *Proc. Nat. Inst. Sci. India*, 1950 *c*, **16**, 157.

——— and Majumdar, G. P... "The leaf-base and the internode: their true morphology," *The Palæobotanist*, 1952, **1**, 351.

Parkin, J. .. "The stipule considered phylogenetically," *The North-Western Naturalist*, 1948, March-December.

———— .. "The Durian Theory—A Criticism," *Phytomorphology*, 1953, **3**, 80.

Philipson, W. R. .. "The development and morphology of the ligule in Grasses," *New Phyt.*, 1935, **34**, 310.

Saha, B. .. "Morphology of the ligule and sickle of *Oryza sativa*," *Bull. Bot. Soc. Beng.*, 1952, **6**, 49.

Saunders, E. R. .. "The Leaf-Skin Theory of the Stem," *Ann. Bot.*, 1922, **36**, 135.

Sharma, P. .. "Stipules, peduncles and axillary buds of *Mutingia calabura*," (unpublished).

Sharman, B. C. .. "Developmental anatomy of the shoot of *Zea mays*," *Ann. Bot. N.S.*, 1942, **6**, 245.

Sinnott, E. W. .. "The anatomy of the node as an aid in the classification of Angiosperms," *Amer. J. Bot.*, 1914, **1**, 303.

——— and Bailey, I. W. .. "Investigations on the phylogeny of Angiosperms: III. Nodal anatomy and the morphology of stipules," *ibid.*, 1914, **1**, 441.

———— .. "Investigations on the phylogeny of Angiosperms: V. Foliar evidence as to the ancestry and early climatic environment of the Angiosperms," *ibid.*, 1915, **2**, 1.

Swamy, B. G. L. .. "Further contributions to the morphology of the Degeneriaceæ," *J. Arnold. Arb.*, 1949, **30**, 10.

Tyler, A. A. .. "The nature and origin of stipules," *Ann. N. York. Acad.*, 1897, **10**, 1.

Vines, S. H. .. *An Elementary Text-Book of Botany*, London, 1910.

Willis, J. C. ..*A Dictionary of the Flowering Plants and Ferns*, Cambridge, 1951.

* Literature marked with an asterisk has not been seen in original.

SPONTANEOUS FEEDING RESPONSE TO COLOURS IN *PAPILIO DEMOLEUS* L.

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Introduction

Early in 1910, August Forel wrote: "Die Farbe bildet ein Merkzeichen, aber keine Anziehung, an und für sich, für das Insekt" (Forel, 1910). By that he meant that for insects the colour forms a signal by which to remember a flower, but in itself is not attractive to them. However, Knoll (1921, 1922, 1926) tested a few individuals of the hawk-moth *Macroglossum stellatarum* with some coloured papers belonging to the Hering series and found that even freshly emerged imagines of this hawk-moth, who could not have had any experience with colour, showed definite responses to colours.

These results obtained by Knoll with the hawk-moth and those obtained by Ilse (1928) with butterflies, disprove Forel's statement quoted above. Thus, it has now certainly been established that some insects have an inborn colour preference.

During our investigation of the biology of the citrus pest, *Papilio demoleus* (Lepidoptera), we also tried to find out the part, colour plays in their imaginal life in different physiological states.

Material and Method

The specimens used for these experiments were reared from eggs collected previously on citrus plants.

The experiments were performed in a special 'experimental part' of a large insect observation cage which had been erected for that and similar purposes. This part measured 12′ in length, 12′ in breadth and 6½′ in height. It had an inner lining of mosquito-netting in addition to the outer galvanised iron wire-netting of ½″ mesh supported by wooden framework. The experimental part was kept devoid of any coloured object apart from those specially provided for the experiments.

Artificial flowers were prepared from the Standard Saturated Ostwald series of 24 colours. Each flower was about 2″ in diameter. These flowers

were mixed with similar flowers prepared from grey papers of various shades from the Bauman Grey series, so as to decide whether the insects were attracted to the hue and not to the particular shape or particular degree of brightness. Each flower was provided with a central glass test-tube with $\frac{1}{2}''$ diameter. No food (sugar water) was offered in these tubes throughout the course of experiments. The flowers were arranged by inserting these test-tubes through a wire-netting of $\frac{1}{2}''$ mesh held horizontally in a wooden frame, similar to a common picture frame.

The Ostwald series has 24 hues or colours starting from yellow and ending in yellowish green, *via* orange, red, purple, blue, blue-green and green. Each of these is indicated by a numeral, *e.g.*, numbers 7 and 14 represent spectrum-red and spectrum-blue colours respectively. Added to the number are letters indicating the degree of saturation, *e.g.*, *pa* 7 is saturated spectrum-red and *pa* 14 is saturated spectrum-blue.

On account of the limited field of vision to be expected in such small insects like butterflies, all the 24 papers were not presented simultaneously. Consequently, 3 different experiments were performed by subdividing the series of Ostwald colour papers into 3 groups of 8 papers each. Each group in itself covered the whole range of colours as can be seen from Table I.

TABLE I

Distribution of the coloured papers of the Standard Ostwald series in respective experiments

Colours	Ostwald Papers		
	Expt. I	Expt. II	Expt. III
Yellow	*pa* 2	..	*pa* 1
Orange-yellow ..	..	*pa* 3	..
Orange	*pa* 4	..	*pa* 5
Red	*pa* 7	*pa* 6	..
Reddish-purple ..	..	..	*pa* 8
Purple	*pa* 10	*pa* 9	*pa* 11
Blue	*pa* 13	*pa* 12 *pa* 15	*pa* 14
Blue-green	*pa* 16 *pa* 19	*pa* 18	*pa* 17
Green	..	*pa* 21	*pa* 20
Yellowish-green ..	*pa* 23	*pa* 24	*pa* 22

For each experiment 25 flowers were taken which included 2 from each of the 8 hues or colours of the Ostwald series and 9 different grey shades of the Bauman series. These 25 flowers were arranged horizontally in 5 rows of 5 flowers each. This arrangement was offered to the inexperienced unfed butterflies and their visits on each of the coloured papers were counted separately.

The possibility that the butterflies might be attracted to a coloured paper due to its particular position in the whole arrangement was minimised by offering 2 models of each colour and furthermore, almost excluded by frequently turning the whole arrangement through 90° or 180° during the course of the experiments.

Observations and Results

When the arrangement of papers was offered, the hungry butterflies showed a definite feeding reaction, that is, each one approached the artificial flower in flight, landed on it, unrolled the tongue and performed probing and sucking movements with it on the coloured surface. These movements have previously been observed and described in other butterflies (Kühn and Ilse, 1925).

The results of these three experiments are represented by Tables II, III and IV, and by graphs I, II and III respectively.

Table II

Analysis of the results of Experiment I

Ostwald Colour	No. of Visits	% of Visits	Total No. of Visits on Colours	Total No. of Visits on Greys
pa 2	5	1·9		
pa 4	17	6·6	252	5
pa 7	8	3·1		
pa 10	69	26·8		
pa 13	82	31·9		
pa 16	66	25·7		
pa 19	5	1·9		
pa 23	1	0·4		

TABLE III

Analysis of the results of Experiment II

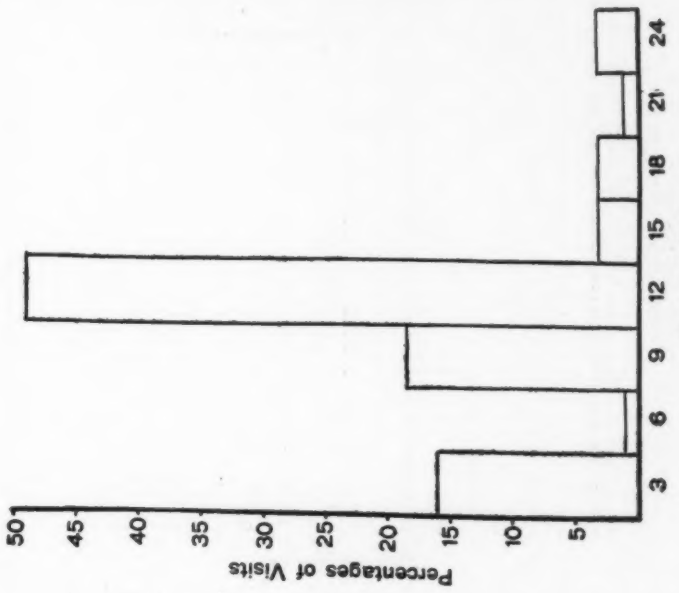
Ostwald Colour	No. of Visits	% of Visits	Total No. of Visits on Colours	Total No. of Visits on Greys
pa 3	26	16·5		
pa 6	1	0·6	151	7
pa 9	29	18·4		
pa 12	78	49·4		
pa 15	5	3·2		
pa 18	5	3·2		
pa 21	2	1·3		
pa 24	4	2·5		

TABLE IV

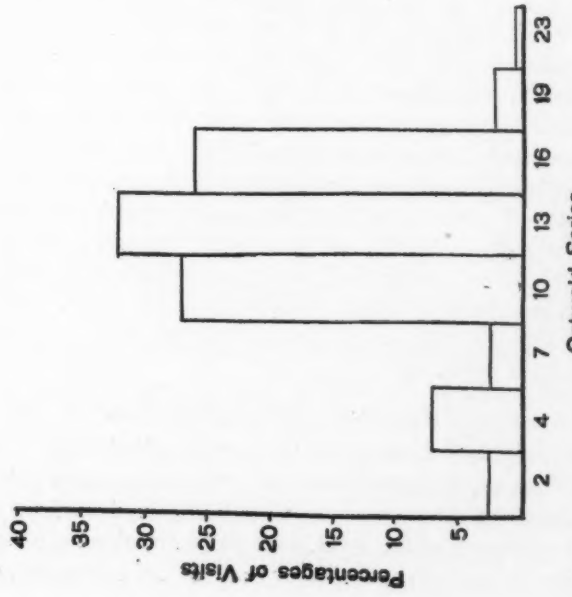
Analysis of the results of Experiment III

Ostwald Colour	No. of Visits	% of Visits	Total No. of Visits on Colours	Total No. of Visits on Greys
pa 1	5	2·0		
pa 5	14	5·6	237	13
pa 8	27	10·8		
pa 11	76	30·4		
pa 14	98	39·2		
pa 17	12	4·8		
pa 20	1	0·4		
pa 22	4	1·6		

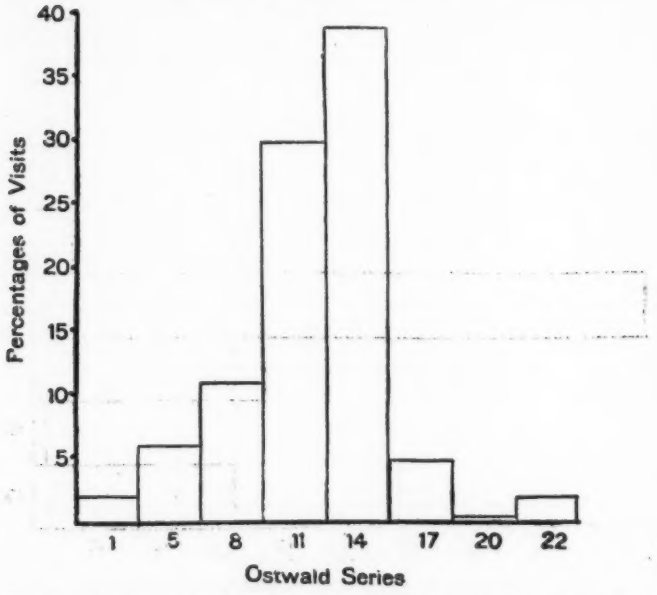
A combined graphic representation showing each of the results of these three experiments (Graph IV) shows that for *Papilio demoleus* the blue and purple colours are most attractive throughout. It has to be kept in mind that the result of each of the three experiments is not directly comparable to that of the two others, because in each of these part-experiments the number of strongly attractive colours varies slightly. If a greater number of very



GRAPH II. Spontaneous feeding responses i n *Papilio demoleus*. Experiment II.



GRAPH I. Spontaneous feeding responses in *Papilio demoleus*. Experiment I.

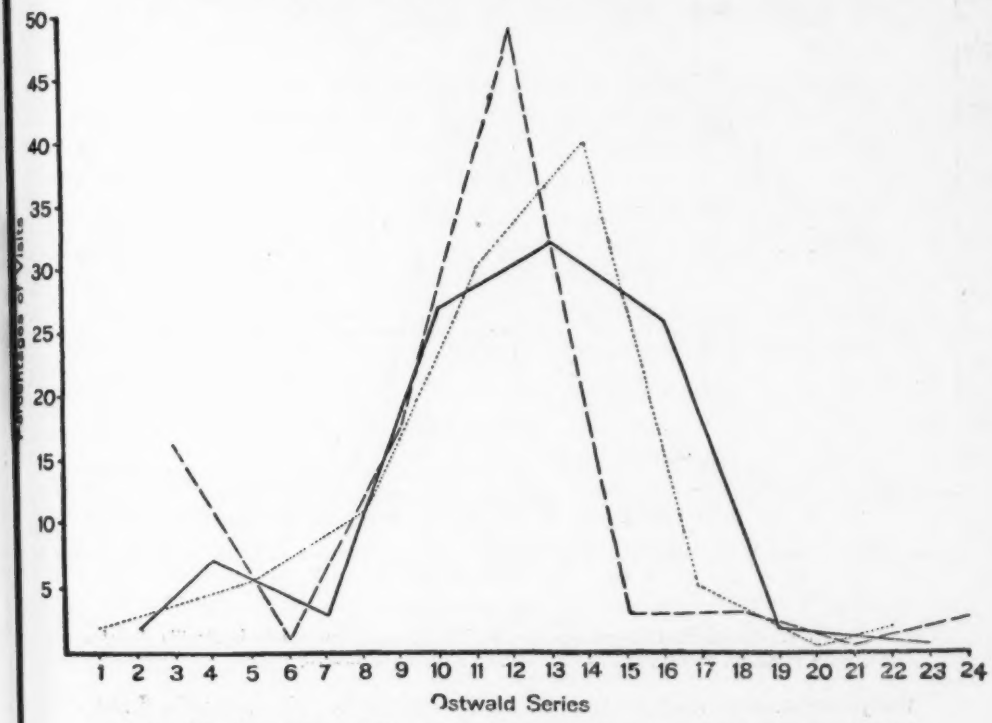


GRAPH III. Spontaneous feeding responses in *Papilio demoleus*. Experiment III.

attractive colours is present, the rest of the colours are comparatively neglected. For this reason, we have not constructed a compound graph, using the percentages of the visits in each case, but have simply superimposed the three graphs on one sheet. From this representation, though we cannot conclude details, the general trend becomes very clear. The attractiveness increases from *pa* 8 (purple), reaches peak values at *pa* 12 (violet), *pa* 13 (blue) and *pa* 14 (blue), and again decreases at *pa* 17 (blue-green). The surprisingly low percentage of visits on *pa* 15 (blue) may be due to the simultaneous presence of such highly attractive colour as *pa* 12 (violet) in the same set. The yellow, yellowish-green, green and blue-green colours (*pa* 2, *pa* 1, *pa* 24, *pa* 23, *pa* 22, *pa* 21, *pa* 20 and *pa* 19) are comparatively neglected. Moreover, of the grey shades only the brightest, *i.e.*, almost white ones were visited while other grey shades were neglected.

DISCUSSION AND CONCLUSION

Schremmer (1941) working on the sensory physiology of the day-flying moth *Plusia gamma* found that freshly emerged moths of this species were unable to find food only by means of optical stimulus of the colour of the flowers in the absence of the scent. Only when, by chance, it has found food on a scentless flower, will it revisit objects of that colour when in search of



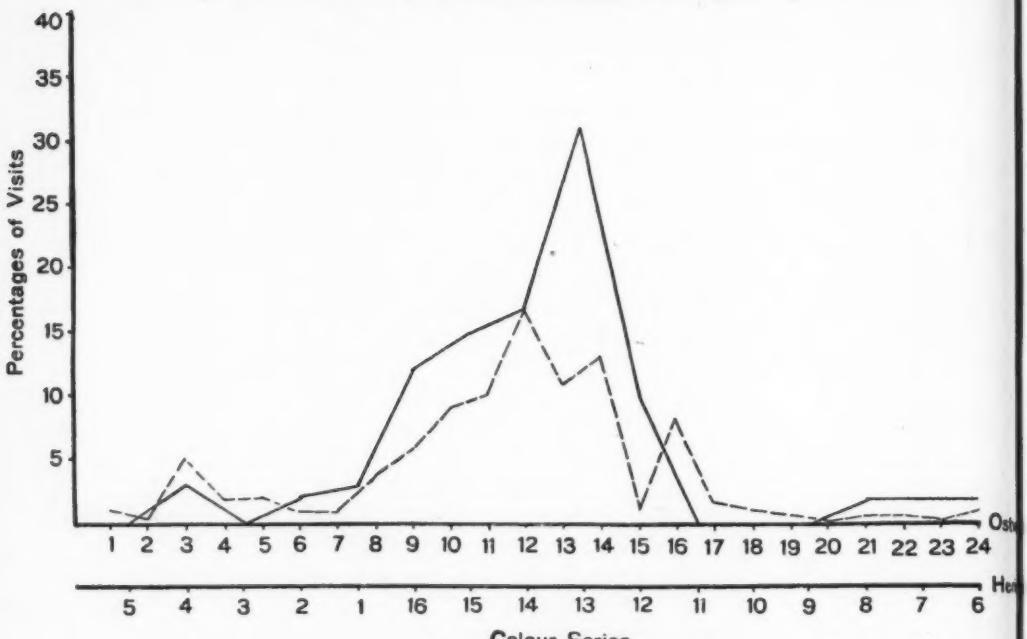
GRAPH IV. Comparison of the results of three experiments on the Spontaneous feeding responses in *Papilio demoleus*. Expt. I = ———, Expt. II = - - - - & Expt. III = ...

food. But even this association of food and colour does not last long. Thus, *Plusia gamma* shows no inborn preference to colours (Forel's opinion still holds for insects like *Plusia gamma*).

On the other hand, specimens of *Papilio demoleus* devoid of any previous experience with colour, in their feeding responses show a marked 'inborn' preference to blue and purple colours. They are 'spontaneous' reactions, or in Pavlov's terminology, chains of 'unconditioned reflexes'.

The spontaneous feeding reaction to coloured papers by the European Swallow-tail *Papilio machaon*, investigated by Ilse (1928) by means of the Hering series of coloured papers is very similar to that given by *Papilio demoleus* (in this case depicted by a compound graph) (Graph V). Both the species prefer blue and purple colours when they are in the feeding state.

From the point of view of comparative physiology, this result is of great interest. It has been found that primitive insects show more preference for



GRAPH V. Comparison of spontaneous feeding responses to colours in *Papilio demoleus* and *Papilio machaon*. *P. demoleus* = - - - - & *P. machaon* = ——.

the yellow colour when they are in the feeding state. For example, flower-visiting Diptera (Ilse, 1949; Kugler, 1951), Aphids (V. Moericke, 1950 and 1955) and also the white-flies Aleyrodes (Weber, 1931) respond to yellow colour when they are in the feeding state. Even among butterflies, only the Pierids have the same type of colour preference as the Papilios, while the comparatively primitive ones like the Nymphalid *Vanessa polychlorus* show the highest peak in yellow and only a small one in blue. The graphs for the inborn colour preference for other Nymphalids, *e.g.*, *Vanessa io* (Peacock) and *Vanessa urticæ* (Ilse, 1928), and *Eumenis semele* (Tinbergen *et al.*, 1942) show 2 peaks, one in yellow and the other in blue, both of almost equal heights.

It is hoped that this investigation of the spontaneous colour response by *Papilio demoleus* in the feeding state might, in future, be useful in devising control measures for this pest on citrus.

SUMMARY

1. Freshly emerged imagines of *Papilio demoleus*, which had no previous experience with colour, were kept in a large cage devoid of any coloured object apart from those specially provided for the experiments.

2. These were offered artificial coloured flowers prepared from the standardized Ostwald series.

3. On the coloured flowers, the inexperienced unfed specimens showed a characteristic feeding response: they approached the artificial flower in flight, landed on it and unrolled their tongues with which they performed probing and sucking movements on the paper.

4. The results clearly show that *Papilio demoleus* in the feeding state, is mainly attracted to the blue and purple colours while the yellow, yellowish-green, green and blue-green colours are completely neglected.

5. Thus, they have provided a proof that certain insects, contrary to Forel's assumption (1910), show not only acquired but also inborn or spontaneous preferences for colours.

References

Forel, A. .. *Das Sinnesleben der Insekten*, München, 1910.

Isle, D. .. "Über den Farbensinn der Tagfalter," *Z. vgl. Phys.*, 1928, **8**, 658–691.

———— .. "Colour Discrimination in the Dronefly *Eristalis tenax*," *Nature*, 1949, **163**, 255.

Knoll, F. .. "Insekten und Blumen," *Abh. d. zool. bot. ges. Wien*, 1921, 1922, and 1926, **12.**

Kugler, H. .. "Die Blütenökologische Untersuchungen mit Goldfliegen (Lucilien)". *Ber. dtsch. bot. Ges.*, 1951, 64.

Kühn, A. and Ilse, D. .. "Anlockung von Tagfaltern durch Pigmentfarben," *Biol. Ztrbl.*, 1925, 45.

Moericke, V. .. "Über das Farbsehen der Pfirsichblattlaus *Myzodes persicæ* Sulz.," *Zs. f. Tierpsychol.*, 1950, Band **7**, Heft 2.

———— .. "Über die Lebensgewohnheiten der geflügelten Blattläuse (Aphidina) unter besonderer Berücksichtigung des Verhaltens beim Landen," *Ibid.*, 1955, Band **37**, Heft 1.

Schremmer, F. .. "Sinnesphysiologie und Blumenbesuch des Falters von *Plusia gamma* L.," *Zool. Jb. Abt. Syst.*, 1941, **74**, 375–434.

Tinbergen, N., Meeuse, B. J. D., Boerema, L. K., and Varossieau, W. W. "Die Balz des Samfalters *Eumenis* (*Satyrus*) *semele* L." *Zs. f. Tierpsychol.*, 1942, Band **5**, Heft 1.

Weber, H. .. "Biology of *Trialeurodes*, Hem.," *Z. Morph. Oekol. Tiere.*, 1931, **23**, 575–753.

OBSERVATIONS ON WING DEVELOPMENT IN APHIDS

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Little experimental work has been done in India on the problem of Wing Development in Aphids, although several workers notably, Davidson (1914), Brittain (1921), Mason (1922–23), Davidson (1921), Davidson (1929), Rivnay (1937) and Frank Wilson (1938) from the West have published considerable information on the subject. Preliminary observations on this particular aspect of the life-cycle of aphids were made by the author by arranging a series of laboratory experiments and field observations during 1945 to 1948. Subsequently, Ratan Lal (1951) published the results of his experiments conducted by him during 1950–51.

Conflicting views on the production of winged forms of aphids have been expressed by several workers. Davidson (1921) and Mason (1922) found that an unsatisfactory feed did not lead to the development of alate-aphids. On the contrary, Davidson (1921 *b*) and Shull (1929) concluded that deterioration in the condition of the host, as a result of overcrowding by aphids, produced the alate forms. Later, Schæfer (1938) inferred that abundance of food led to the development of alate forms. He further observed that a distinct correlation existed between overcrowding of aphids, small size of the plant and the development of alate forms. In the meantime Ackerman (1926) and Reinhardt (1927) had tried to show that winged forms of aphids when overcrowded or starved produced comparatively less alate aphids. The present paper gives a brief account of the development of winged forms in *Macrosiphum jaceæ*, based on laboratory and field observations made during 1945 to 1948.

II. Technique

The insects were reared on potted seedlings of *Carthamus tinctorius* and covered over with lamp chimneys, capped with fine muslins. To maintain uniformity, the progeny of the same parent maintained on a stock plant was utilised for the experiments. For estimating population counts of

aphids in the field, the method adopted by Gray and Schah (1941) was followed with suitable modifications.

III. Life-History of *M. jaceæ*

Macrosiphum jaceæ is the common black aphid on safflower plants during October to March. In the early part of the season when the host plants are about 3″ to 4″ in height, winged forms of *M. jaceæ* are commonly observed in the fields. The average mean temperatures during the early part of October range between 68° F. to 78·2° F., and the atmospheric humidity varies between 60·0 to 78·2%. The succeeding generations of aphids normally consist of apterous viviparous females with very low proportions of winged forms in the colony. Once more, the winged forms begin to appear in larger proportions by the end of January. Later, as the vegetative growth of plants ceases and the host plants become woody and fibrous the winged forms increase in number and finally migrate to other suitable hosts. The increase in the population of alatæ on the host is normally accompanied by a gradual trend in the rise of average mean temperatures. Practically a similar state of things occurs during the seasonal histories of other aphids. It is interesting to investigate, therefore, the influence of environmental changes on the occurrence of alatæ, as they constitute an essential phase during the seasonal history of aphids.

IV. Effects of Overcrowding on the Presence of Alatae and Apterae

To determine the effects of overcrowding on the production of alatæ of *M. jaceæ*, 15 safflower plants, one in each pot (seeds planted on the same day) were divided into three series of five plants each. In series A, one apterous individual of the premature instar, in series B five individuals and in series C ten individuals were transferred to each plant. The insects belonged to the same generation and all the other treatments were alike in the experiments. The plants were kept under the muslin bags and observations were made from 3–12–1947 to 30–12–1947. At the end of the experiments, the aphids were killed by chloroform and counted for incidence of alatæ and apteræ. The average temperatures for the period under experiment were Max. 85·7° F. and Mini. 48·1° F. (see Table I).

Discussion.—If there are innumerable factors which by their combined action govern the production of alatæ among aphids, overcrowding of aphids on the plants or a portion of it, appears to be one of the major factors. The data reveals that in series B and C the production of alatæ per initial single parent aphid is more as compared to A. At the same time the

TABLE I

Showing the Effects of Population Density on the Development of Alatæ

Series	Date of commencement of the experiment	Initial population per plant	Analysis of population at the end of the experiment		
			Apterous	Nymphs with wing pads	Alatæ (adults)
A	3–12–1947 ..	1	75	2	..
		1	62	..	..
		1	45	..	..
		1	80	6	..
		1	57	1	..
	Average ..	1	63·8	1·8	..
B	3–12–1947 ..	5	212	22	4
		5	201	16	3
		5	167	6	6
		5	168	11	3
		5	184	12	5
	Average ..	1	37·3	2·71	0·8
C	3–12–1947 ..	10	442	32	16
		10	470	28	14
		10	316	15	12
		10	362	27	6
		10	417	19	9
	Average ..	1	40·1	2·4	1·1

Remarks.—Plants were kept healthy throughout the experiment. Apterous forms noted above include adults and nymphs without wing pads. Very small individuals which could not be classified properly were not accounted.

production of apteræ per initial single parent aphid per plant in series B and C is reduced as compared to A. Thus, the experimental findings in a way support the observations of Frank Wilson (1938) and subsequently are in conformity with the conclusions of Ratan Lal (1952).

V. EFFECT OF DARKNESS ON WING DEVELOPMENT

Observations were made by arranging laboratory experiments during 1948 with a view to investigate the effect of darkness on the occurrence of alatæ. 12 safflower seedlings, one in each pot (seeds planted on the same day) were selected and one apterous individual of the premature instar was

transferred to each plant. The trial plants were subjected to increasing hours of darkness by keeping them in dark chamber, beginning with one hour per day in the week beginning, to five hours per day in the final week. In the control treatment the aphids were allowed to breed normally in the laboratory.

TABLE II

Showing the Effects of Darkness on the Development of Winged Forms in M. jaceæ

S. No.	Date of commencement of experiment	Initial infestation per plant	Aphid population at the end of				
			I	II	III	IV	V week
1	12–8–1948 ..	1 Adult	1	2	2	3	3
		9 Nymphs	15	14	20	16	13
2		7 Nymphs	1	1	2	2	4
		1 Adult	18	17	13	6	9
3		1 Adult	1	3	3	5	4
		5 Nymphs	19	16	21	19	16
4		1 Adult	1	2	6	4	4
		8 Nymphs	11	14	9	21	19
5		1 Adult	1	2	4	3	3
		9 Nymphs	21	13	11	16	20
6		1 Adult	1	2	2	3	2
		7 Nymphs	11	6	16	19	19
7		1 Adult	2	2	3	3	3
		7 Nymphs	16	14	11	9	15
8		1 Adult	1	1	2	3	3
		10 Nymphs	6	11	19	22	18
9		1 Adult	2	3	4	6	5
		8 Nymphs	4	16	19	11	10
10		1 Adult	2	2	3	3	2
		8 Nymphs	6	14	22	19	12
11	Control ..	1 Adult	1	3	4	5	6
		9 Nymphs	21	22	16	30	44

The average mean temperatures of the room during the experiment ranged between 72·1° to 75·3° F.

Discussion.—None of the youngones developed wing pads during the period of five weeks beginning from 12–8–1948 to 9–9–1948. It is evident that subjection of aphids to darkness may not stimulate wing formation in aphids.

VI. SUMMARY

An account of the results of experiments regarding overcrowding of aphids on plants and subjection of aphids to darkness as factors in the wing development of aphids *Macrosiphum jaceæ* is given.

(1) It is observed that poor nutrition due to overcrowding induces to a large extent wing development in aphids.

(2) Subjection of aphids to increasing hours of darkness did not produce winged forms of aphids.

REFERENCES

1. Davidson, J. .. *Ann. appl. Biol.*, 1914, **1**, 118–41.
*2. Brittain, W. H. .. *Proc. Canadian ent. Soc.*, 1921, **7**, 7–29.
*3. Mason, A. C. .. *Florida Ent.*, 1922, **6**, 25–32.
4. Davidson, J. .. *Proc. Roy. Dublin Soc. Sci.*, 1921 *a*, **16**, 304–22.
5. ———— .. *Ann. appl. Biol.*, 1921 *b*, **8**, 51–65.
6. Ackerman, L. .. *J. expt. zool.*, 1926, **44**, 1–61.
7. Reinhardt, H. J. .. *Bull. Texas agric. expt. sta.*, 1927, **353**, 1–19.
*8. Shull, A. F. .. *Biol. Rev. and proc. Cambridge Phil. Soc.*, 1929, **4**, 218–48.
9. Rivnay, E. .. *Bull. ent. Res.*, 1937, **28**, 173–79.
*10. Schaefer, C. W. .. *J. agric. Res. Wash.*, 1938, **57**, 825–41.
11. Frank Wilson .. *Trans. ent. Soc. Lond.*, 1938, 87.
12. Gray, K. W. and Schah, J. *J. econ. Ento.*, 1941, **30**, 411.
13. Ratan Lal .. *Indian J. Ent.*, 1952, **14** (1), 11–19.

*Original references could not be referred.

CYTO-TAXONOMIC EVIDENCE FOR THE AFFINITY BETWEEN *CAJANUS INDICUS*, SPRENG. AND CERTAIN ERECT SPECIES OF *ATYLOSIA* W. & A.

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I. Introduction

The genera *Cajanus* D.C. (*Cytisus*, Linn.) & *Atylosia* W. and A. belong to the sub-tribe *Cajaneæ* of the tribe *Phaseoleæ* of the *Papilonaceæ*. The main distinguishing feature between *Cajanus* and *Atylosia* is the presence of arilate seeds bearing large conspicuous divided strophiole in the latter. The species included in both these genera have more or less common range of distribution extending over Australia, Mauritius, tropical Africa and Asia, India, Indo-China and some other Pacific islands. Index Kewensis mentions about 30 species of *Cajanus* with predominant distribution in Australia but only six of these are valid and the rest are synonyms of species reported by various authors under the genera *Atylosia*, *Argyrolobium*, *Rhynchosia*, *Glycine*, *Astragalus*, *Desmodium* and *Calopogonium*.

Index Kewensis also mentions over 35 species of *Atylosia* distributed in India, China, Burma, Malaya and Australia. A few of these are synonyms of species reported under the genera *Cajanus*, *Rhynchosia* and *Dunbaria*.

II. Diversity of Forms of *Cajanus indicus* Spreng. in India

Cajanus indicus Spreng. (*Cytisus cajan* Linn., *Cajanus flavus* D.C., *C. bicolor* DC, *C. pseudo-cajan* Jacq., *Cajanus cajan* Mills) is an extensively cultivated pulse crop of India. de Candolle attributes to it an origin in Tropical Africa and Madagascar, from where it is believed to have been introduced into India over 3,000 years ago. However, the genus does not show expected range of variation in Africa and according to Engler (1915) there is no definite evidence in favour of its African origin.

Both cultivated and wild forms of this species have been reported from Indo-China (Sampson, 1937). Though not definitely reported

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as wild in India, this crop shows greater variability in India than elsewhere (Sampson, 1937). de Candolle recognized two species *C. flavum* D.C. and *C. bicolor*, mainly based on corolla characters, which were later united into the single species *C. indicus* Spreng. and the above two forms were reduced to a mere varietal status (Watt, 1889). Krauss (1927) and later Shaw *et al.* (1933) recognized two primary types (*a*) a short, early peninsular type and (*b*) a tall late *Arhar* type from Northern India, tending to perennial habit. Central India has both these types. Shaw *et al.* (1933) isolated 107 types from samples collected in various parts of India while Mahta and Dave (1931) isolated 36 types from Madhya Pradesh alone. This is indicative of the very great variability of this species in India.

III. Possibility of Presence of Wild Prototypes in India

In view of great diversity of *Cajanus indicus* Spreng. in India, it was thought that some related wild prototypes may be present in India itself. As there is only one species of *Cajanus* reported from India, a search was made among the closely related genera and species of the sub-tribe *Cajaneæ* in India. It was observed that morphologically, the genus *Cajanus* very closely resembles the genus *Atylosia*. The main distinguishing feature, on the basis of which these two genera are separated, is the presence of arilate seeds with large conspicuous, divided strophiole in *Atylosia*. Comparative observations on some cultivated forms of *Cajanus indicus* and species of *Atylosia* revealed that this point of distinction is not very marked and that the degree of conspicuousness of strophiole in some *Cajanus* types and some species of *Atylosia* was more or less of the same order. Species of *Atylosia* have either erect or twining stems and the former come closest to *Cajanus*, morphologically. Such species of *Atylosia* with erect stem include *A. lineata* W. and A., *A. sericea* Benth., *A. candollei* W. and A., *A. geminiflora* Dalz and, *A. mollis* Benth., etc. The names of some of the species of *Atylosia* such as *A. cajanifolia* Haines (Orissa), and *A. cajanoides* Cordem (Reunion, Bourbon), are indicative of such similarity among some of the species placed in the genera *Cajanus* and *Atylosia*.

IV. Investigations on Some Erect Species of Atylosia from Western Ghats

Atylosia lineata W. & A. and *A. sericea* Benth. are widely distributed in Western Ghats. They were collected from Mahabaleshwar, Panchgani, Khandala, Lonavala, Matheran, Dang Forests and other locations in Western Ghats during 1949. Both in respect of vegetative characters, physiognomy and general growth habit, these two species show marked resemblance with cultivated types of *Cajanus indicus*. In fact, both these species of *Atylosia*

are locally called by the farmers and aboriginal tribes as *Ran Tur* (wild Tuar) or wild forms of *Cajanus indicus* Spreng., and are consumed by the local tribes as a substitute grain pulse. For this purpose, it is interesting to note that *Atylosia lineata* W. & A. is synonymous with *Cajanus lineatus* Graham, so that obvious botanical affinity between this species and *Cajanus*, has received recognition from botanists.

V. Chromosome Numbers and Comparative Chromosome Morphology

In view of such indications of close affinity between *Cajanus indicus* Spreng. and *Atylosis lineata* W. & A. and *A. sericea* Benth., chromosome numbers were determined. Chromosome numbers of *Cajanus indicus* Spreng. have been already reported as $n = 11$ and $2n = 22$ (Roy, 1933). It was found that both *A. lineata* and *A. sericea* have also $n = 11$ and $2n = 22$ as their chromosome numbers. The chromosome numbers of these two species are being reported here for the first time. The only other species of *Atylosia* of which the chromosome number has been reported so far is *A. barbata* which also has $2n = 22$ chromosomes (Tschechow and K., 1932).

Comparative chromosome morphology of the somatic metaphase at comparable degree of linear condensation, was studied in each of the three species and the results have been given in Tables I to III (Figs. 1 to 6).

Table I

Morphological analysis of somatic chromosome complement in Cajanus indicus, *Spreng.*

Class	Position of constriction		Length of component segments microns	Total length	Mean diameter	Total No. of chromosomes
	Primary	Secondary				
I	S.M.	S.T.	1·2+1·2+2	4·4	0·5	2
II	S.M.	..	3+1·5	4·5	,,	2
III	Med.	..	2+2	4	,,	2
IV	S.M.	..	2·5+1·2	3·7	,,	2
V	Med.	..	1·7+1·7	3·4	,,	4
VI	S.M.	..	2+1·2	3·2	,,	2
VII	S.M.	..	2+1	3	,,	2
VIII	Med.	..	1·5+1·5	3	,,	2
IX	S.M.	..	1·7+1	2·7	,,	2
X	Med.	..	1·2+1·2	2·4	,,	2

Total number of chromosomes .. 22

Total length of complement ,, 75·4 microns.

TABLE II

Morphological analysis of somatic chromosome complement in Atylosia lineata *W. & A.*

Class	Position of constriction		Length of component segments microns	Total length	Mean diameter	Total No. of chromosomes
	Primary	Secondary				
I	S.T.	..	4+1	5	0·5	4
II	Med.	..	2+2	4	,,	4
III	S.M.	..	2·2+1·7	3·9	,,	2
IV	S.M.	..	2·2+1·5	3·7	,,	6
V	S.T.	..	2·5+1	3·5	,,	2
VI	S.M.	..	2+1·2	3·2	,,	2
VII	Med.	..	1·5+1·5	3	,,	2

Total number of chromosomes .. 22

Total length of complement .. 82·4 microns.

TABLE III

Morphological analysis of somatic chromosome complement in Atylosia sericea, *Benth.*

Class	Position of constriction		Length of component segments microns	Total length	Mean diameter	Total No. of chromosomes
	Primary	Secondary				
I	S.M.	S.T.	1·7+1·7+1	4·4	0·5	2
II	S.T.	..	3·5+1	4·5	,,	2
III	S.M.	..	3+1·5	4·5	,,	2
IV	S.T.	..	3+1	4	,,	2
V	Med.	..	2+2	4	,,	2
VI	S.M.	..	2·5+1	3·5	,,	4
VII	S.M.	..	2+1·2	3·2	,,	2
VIII	Med.	..	1·5+1·5	3	,,	6

Total number of chromosomes .. 22.

Total length of complement .. 81·2 microns.

It will be seen from the foregoing Tables I to III as also idiograms of the three species (Figs. 4 to 6), that the longest pair I is homo-morphic in *C. indicus* and *A. sericea*, with primary sub-medium and secondary sub-terminal constrictions. Pair I in *A. lineata* differs in slightly greater length and

absence of sub-median constriction. Pair II is almost similar both in *A. lineata* and *A. sericea*, with sub-terminal constriction. In the case of *Cajanus*, pair II has sub-median constriction. Pairs III, VII and X are homo-morphic in *Cajanus* and *A. lineata*. Pairs I, IV, V, VIII and IX are homo-morphic in *Cajanus* and *A. sericea*. Pair XI is similar in all and pair VI is dissimilar in all the three species. Therefore, *Cajanus indicus* has six pairs apparently similar to those in *A. sericea* and four pairs apparently similar to those in *A. lineata*. Thus, purely on the basis of apparent chromosome morphology, *A. sericea* seems to be more closely related to *Cajanus indicus*. This tentative inference must be confirmed from relative fertility of the first hybrid generations and genetic studies on the segregating generations.

It is interesting to note that the total length of somatic chromosome complement at metaphase is almost the same in three species with very little difference. A part of this observed difference may also be due to the fact that the metaphase plates studied in three species may not be at corresponding degrees of linear condensation which varies with advancing metaphase.

From the above data it appears that structural changes may have played a major role in the origin of differentiation between *Cajanus* and *Atylosia*-species.

VI. Evidence from Hybridisation Experiments

One of the authors (G. B. D.) collected the two *Atylosia* species and planted them along with the cultivated *Cajanus indicus* Spreng., in the Experimental Gardens of the Economic Botany Section, at the College of Agriculture, Poona, in the year 1949, where he was working then, with a view to undertake their hybridisation. Continuation of this work, initiated by one of the authors, by his successors and co-workers, (Oak, De Cruz, Thombare *et al.*, under the supervision of Prof. Kumar), has resulted in the synthesis of a hybrid between *Cajanus indicus* and *Atylosia lineata*. The hybrid shows considerable fertility and seed setting indicative of high degree of homology among the chromosome complements of *Cajanus indicus* and *A. lineata*.

Two natural hybrids have also been detected among plants raised from seeds of *A. lineata* grown in the Experimental Gardens of the M.A.C.S. Biological Laboratories. Characters of these hybrids are intermediate between *Cajanus indicus* and *A. lineata*. Detailed cytogenetic observations on these hybrids will be presented later.

VII. Evidence from Parallel Mutations

A further support for the affinity of the two species of *Atylosia* with *Cajanus indicus* is provided by the fact that most of the mutant characters

reported in cultivated *Tur* crop so far, are to be found as regular normal characters in some of the *Atylosia* species. Thus for instance, Deshpande and Jeshwani (1952) as also Chaudhari and Patil (1953) have reported a " Creeping" mutant in *Tur* having a weak prostrate stem—a character present in several *Atylosia* species. Pandya, Patil and Chaudhari (1954) have also reported *Tur* mutant with " obovate " leaflets having rounded base and apex which are found in *A. lineata*, *A. candollei*, *A. rugosa* and other species of *Atylosia*. Singh (1942) as also Pandya, Patil and Chaudhari (1954) have reported a mutant with " obcordifolia " leaflets which has been named as *Cajanus obcordifolia*. This character with depressed margin at the tip is found in *Atylosia nivea* from Burma, a specimen of which is available in the Herbarium of the Economic Botany Department of the Agriculture College, Poona. The same authors have described mutants having mucronate apex as in *A. lineata* and tomentose epidermis with profuse silky hairs on the lower leaf surface, as in *A. sericea*. The normal plants of *Tur* (*C. indicus*) have lanceolate leaflets with acute base and apex as in many *Atylosia* species. Therefore, many of the normal as well as mutant characters in cultivated *Tur* (*Cajanus indicus*) are found in one or more species of *Atylosia*. These facts together with other evidence presented above, provides strong support in favour of the close affinities of certain species of the two genera. Additional evidence for the affinities between the species belonging to the two genera will be presented elsewhere.

VIII. SPECIES OF *Atylosia* HIGHLY RESISTANT TO SOME OF THE DISEASES AFFECTING CULTIVATED TUR CROP

The cultivated *Tur* (*Cajanus indicus*) is highly susceptible to the pod-borer insect (*Exelaster atomosa*, W.) and the wilt disease caused by *Fusarium udum* Butler. Considerable quantities of pods of the two *Atylosia* species collected from various locations in Western Ghats, as also the actual field observations revealed extremely rare incidence of pod-borer attack which was altogether absent in some cases. This possibly indicates immunity or very high degree of resistance to pod-borers in these two *Atylosia* species. This highly tentative inference needs confirmation.

Seeds of *A. lineata* and *A. sericea* collected from various locations during 1949–50 in Western Ghats were sown under optimum conditions in glass-house in earthen pots containing soil infected with the culture of *Fusarium udum* made available by the Plant Pathologist to the Government of Bombay. Triplicate trials were repeated twice in the year 1949–50, which showed very high degree of resistance to *Fusarium udum* approaching 97%.

IX. Economic Importance of the *Atylosia* Species

From the foregoing, the importance of certain species of *Atylosia* for breeding superior strains of cultivated *Tur* crop in India will be obvious. Such hybridisation programme will enable us to isolate improved strains of *Tur* combining hardiness, perennial growth habit, tolerance to summer drought and high degree of resistance to certain pests and diseases. In spite of the wide range of variability of cultivated forms, due to occurrence of only a single species of *Cajanus* in India, any scope for further improvement of this crop was limited. The close affinity of *Cajanus indicus* with certain species of *Atylosia* as revealed in the present work provides considerable scope for further improvement of this crop.

Besides the utility of *Atylosia* species for breeding superior strains of cultivated *Tur* crop in India, they may also hold considerable promise as hardy perennial, fodder legumes. They may be grown in mixture with various fodder grasses in suitable proportions so as to provide well balanced nutrition to cattle directly grazing on such pastures. *Atylosia* species are tender and succulent and provide highly palatable leguminous fodder to cattle during their pre-flowering stage. After their root system has deeply anchored in the soil, they are able to withstand repeated cutting or direct grazing. They are perennial and can sustain severe summer drought even on poor eroded soils. They are useful for reclaiming eroded waste lands and for various soil conservation measures.

X. Discussion and General Remarks

Close affinity of the two species of *Atylosia* with *Cajanus indicus* is indicated on morphological, taxonomical and cytogenetic grounds as also from the homology of normal and mutant characters in the two genera and the high fertility of hybrids. If all the evidence from various sides is considered together, it may be justified to include some of the *Atylosia* species, especially those with erect stems, under the genus *Cajanus*. This question will be considered in detail elsewhere.

XI. Summary

A close affinity of *Cajanus indicus* Spreng. with certain erect species of *Atylosia* particularly *A. lineata* W. & A. and *A. sericea* Benth., is indicated on morphological, taxonomical and cytogenetical grounds as also from the homology of some normal and mutant characters in the two genera and high fertility of certain inter-generic hybrids. *A. lineata* and *A. sericea* have the same chromosome numbers as *Cajanus indicus* ($2n = 22$). *A. lineata* and

A. sericea are highly resistant to *Tur* pod-boarer (*Exelaster atomosa*, W.) and *Tur* wilt disease caused by *Fusarium udum* Butler.

Besides the utility of *Atylosia* species as a soil conserving leguminous forage cover crop, they can be utilised for evolving superior strains of cultivated *Cajanus indicus* combining hardiness, perennial growth habit, tolerance to summer drought on poor eroded soils and high degree of resistance to certain pests and diseases.

Occurrence of only a single reported species of *Cajanus* in India imposes certain limitations on the scope for its improvement through breeding. The evidence regarding its close affinity with certain erect species of *Atylosia* opens fresh avenues for breeding and improvement of *Tur*, which is one of the most important grain pulse crops of India.

In the light of evidence for their close affinity, it is suggested that erect stem species of *Atylosia* may be incorporated in the genus *Cajanus* and the two genera may be taxonomically revised.

XII. ACKNOWLEDGEMENT

These investigations were conducted partly at the Economic Botany Section of the College of Agriculture, Poona, and also at the Biological Laboratories of the Maharashtra Association for the Cultivation of Science, Law College Buildings, Poona. Authors are deeply indebted to Prof. L. S. S. Kumar, Economic Botanist to the Government of Bombay, under whose supervision this work was initiated and to Prof. S. P. Agharkar, Director of the M.A.C.S., for providing laboratory and library facilities and for valuable suggestions on the taxonomic aspect of the problem. Thanks are also due to Sri. M. M. Payak, for taking photomicrographs and to Sri. Parakhe and Shri Salvi, for their valuable assistance in the field and laboratory.

XIII. LITERATURE CITED

1. Chaudhari, B. B. and Patil, J. A. — *Curr. Sci.*, 1953, **22**, 153.
2. Deshpande, R. B. and Jeswani, L. M. — *Ind. Jour. Genet. and Pl. Breeding*, 1952, **12**, 50.
3. Engler, A. .. — *Pflanzenwelt Afrikas*, 1915, **3** (1), 665-66.
4. Krauss, F. G. .. — *Journal of Heredity*, 1927, **17**, 227.
5. Mahta, D. N. and Dave, B. B. — *Mem. of Dept. of Agri. Ind. Bot. Ser.*, 1931, **19**, 1.
6. Pandya, P. S., Patil, J. A. and Chaudhari, B. B. — *Poona Agri. Col. Mag.*, 1954, **45**, 1.
7. Roy, B. .. — *Ind. Jour. Agri. Sci.*, 1953, **3**, 1908.
8. Sampson, H. C. .. — *Bull. Misc. Inform., Roy. Bot. Gard. Kew*, 1937, **12**, 184.

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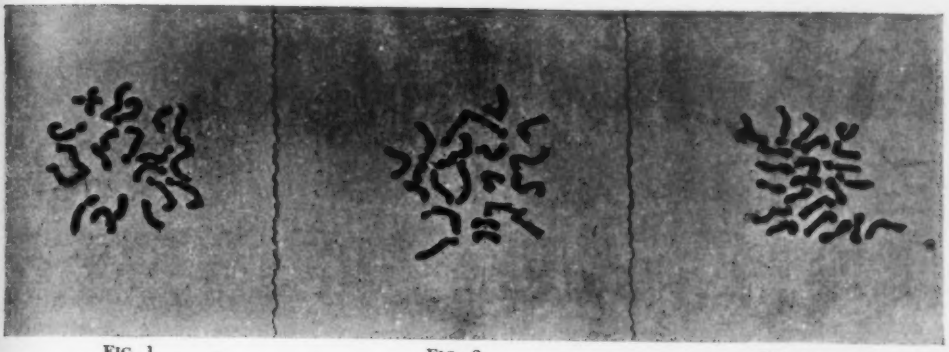

FIG. 1 FIG. 2 FIG. 3

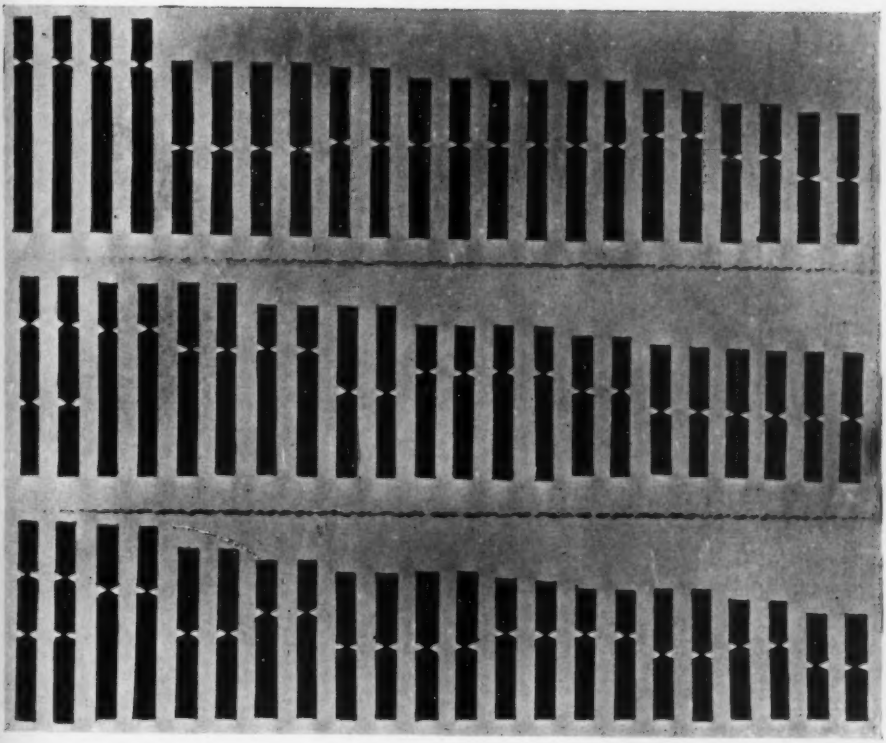

FIG. 4

FIG. 5

FIG. 6

9. Shaw, F. J., Rehman, K. and Singh, H. — *Ind. Jour. Agri. Sci.*, 1933, **3**, 1.
10. Singh, D. N. .. *Ind. Jour. Agri. Sci.*, 1942, **12**, 5.
11. Tschechow, W. and Kartaschowa, N. — *Cytologia*, 1932, **3**, 221.
12. Watt, G. .. *A Dictionary of Economic Products of India*, 1889, **2**, 12.

XIV. Explanatory Notes for Figures

All camera lucida drawings (Figs. 1 to 3) were made at bench level with 90 × apochromatic objective and 25 × compensating eyepiece. Idiograms (Figs. 4 to 6) were drawn at one micron = one centimetre. All originals have been reduced to three-fourths the above size in reproduction.

Explanation of Plate

Figs. 1–6. Fig. 1. Somatic m. phase in *Atylosia lineata* W. & A. $2n = 22$. Fig. 2. Somatic m. phase in *A. sericea* Benth $2n = 22$. Fig. 3. Somatic m. phase in *Cajanus indicus*, Spreng. $2n = 22$. Fig. 4. Idiogram representing somatic complement in *A. lineata* W. & A. Fig. 5. Idiogram representing somatic complement in *A. sericea* Benth. Fig. 6. Idiogram representing somatic complement in *Cajanus indicus*, Spreng.

A BLOOD ANTICOAGULANT FACTOR FROM THE LATEX OF *CARICA PAPAYA*

Part II. Its Nature of Action on Blood Coagulation

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The method of purification and general properties of a blood anticoagulant factor present in the latex of *Carica papaya* were described in an earlier publication (Pillai *et al.*, 1955). Since the factor was found to inhibit effectively the process of coagulation of both plasma and whole blood from different species, it was thought desirable to undertake a detailed investigation to elucidate the probable nature of its inhibitory action on blood coagulation. The process of blood coagulation is a complex reaction brought about by the interplay of a variety of coagulation factors present in the blood. The inhibition of this process of blood coagulation may thus include one or more of the following mechanisms, *viz.*, (1) complex formation with prothrombin preventing its further activation; (2) inhibition of thromboplastic action; (3) inactivation of thrombin and (4) combination with fibrinogen making it incapable of being converted to fibrin.

It was, therefore, found necessary to isolate the different proteins active in blood coagulation and study the effect of the anticoagulant factors on the pure individual components. Similar experiments were carried out with coagulation components prepared from different species.

Experimental

Prothrombin.—Prothrombin was prepared from sheep blood according to the method of Seegers (1952), which consisted of isoelectric precipitation of prothrombin from diluted plasma, adsorption on magnesium hydroxide, elution of the prothrombin by decomposing the latter with carbon dioxide under pressure, fractional precipitation by ammonium sulphate, and later, isoelectric fractionation.

Prothrombin from cow and buffalo plasma was also prepared in a similar manner.

Fibrinogen.—Fibrinogen from cow, sheep and buffalo plasma was prepared by the method of Astrup and Darling (1942). Pure bovine fibrinogen

obtained through the kind courtesy of Prof. W. H. Seegers and Messrs. Parke, Davis & Co., Michigan, was also used.

Thrombin.—Thrombin was prepared from pure prothrombin by using Bacto 2-stage reagent and Bacto Ac-globulin. Both these reagents were generous gift samples obtained from Difco Laboratories, Detroit, Mich.

Russell's viper venom.—Lyophilised venom powder obtained from Haffkeine Institute, Bombay, was diluted with 0·025 M calcium chloride to give a 0·01% solution.

Anticoagulant factor.—The anticoagulant factor was purified by fractional precipitation with acetone (Pillai *et al.*, 1955). A 2% aqueous solution of the dry powder was prepared. For the sake of convenience, the purified anticoagulant factor is designated as AF.

The clotting activity was determined according to the 2-stage procedure of Ware and Seegers (1949). In some experiments Russell's viper venom was used as a source of thromboplastin.

Results

Action of the anticoagulant factor on ideal clotting system

To start with, the action of AF on thrombin activity was determined. The method employed was essentially the same as that described by Ware and Seegers (1949) to determine the prothrombin activity of plasma.

The following solutions were prepared:

A. Bacto 2-stage reagent (containing tissue thromboplastin, imidazole buffer, acacia, sodium chloride and calcium ions).

B. Bacto Ac-globulin. The freeze-dried powder was diluted to 2 c.c. with sterile distilled water. 0·4 c.c. of this solution was further diluted to 60 c.c. with normal saline.

C. To 0·1 c.c. of prothrombin (suitably diluted) were added 2·4 c.c. of diluted Ac-globulin (solution B).

D. 0·1 c.c. of solution C was mixed with 3 c.c. of Bacto 2-stage reagent (A) and incubated for 5 minutes when prothrombin was converted to thrombin.

For determination of thrombin activity the following reaction mixture was compounded.

0·2 c.c. of solution D (thrombin from different species) + 0·2 c.c. distilled water + 0·1 c.c. of fibrinogen from the same species.

The time taken to form a visible clot after the addition of fibrinogen to solution D was taken as the clotting time and was noted with a stop watch. Similarly the coagulation time was measured after the addition of 0·2 c.c. of anticoagulant factor (AF). The results are given in Table I.

TABLE I

Effect of anticoagulant factor on the activity of thrombin prepared from different species

Reaction mixture	Clotting activity
1. Cow thrombin + cow fibrinogen (control)	Clots in 22 sec.
2. Cow thrombin + cow fibrinogen (in presence of AF)	No clotting even after 24 hours. No precipitate formation.
3. Sheep thrombin + sheep fibrinogen (control)	Clotting in 17 sec.
4. Sheep thrombin + sheep fibrinogen (in presence of AF)	No clotting even after 24 hours. No precipitate formation.
5. Buffalo thrombin + buffalo fibrinogen (control)	Clotting in 14 sec.
6. Buffalo thrombin + buffalo fibrinogen (in presence of AF)	No clotting even after 24 hours. No precipitate formation.

Experiments were also carried out with thrombin prepared by incubating prothrombin with Russell's viper venom in calcium chloride solution.

To 0·1 c.c. of suitably diluted prothrombin 0·2 c.c. of venom was added and incubated for 5 min. to convert prothrombin to thrombin. 0·2 c.c. of a 2% solution of AF was added to this and after incubation for 2 min., 0·1 c.c. of fibrinogen was added. Suitable controls were also run without the addition of AF.

In both these cases it was observed that AF effectively inhibited the action of thrombin on fibrinogen. In other words, it interferes with the second stage of blood coagulation.

Since the anticoagulant factor (AF) was found to inhibit thrombin action, different concentrations of AF were added to thrombin as well as pure prothrombin solutions to see whether there will be any interaction between the two.

At the concentration of prothrombin employed for the above clotting experiments, no visible interaction with AF could be detected. But at very high concentrations, the anticoagulant factor formed varying amounts of a white precipitate with both thrombin and prothrombin depending upon the respective concentrations of the reactants.

After inactivation by heating at 85° C. for 30 min., AF lost its property of precipitate formation when added to concentrated solutions of prothrombin.

In another experiment, pure fibrinogen was incubated with a 2% solution of AF for 5 minutes. When this clear solution was added to prothrombin, precipitate formation was retarded to a certain extent. This obviously indicates that although there is no precipitate formation when fibrinogen and AF are mixed, some soluble complex is formed so that further interaction between AF and prothrombin is retarded. When prothrombin is added to the mixture, possibly dissociation of the fibrinogen-AF complex occurs and the free AF generated forms a precipitate with the added prothrombin.

The ability of AF to form a thick precipitate with high concentrations of prothrombin suggests that it might form a soluble complex with prothrombin in dilute solutions usually employed in the 2-stage procedure of Ware and Seegers (1949), and thereby interfere with the first stage as well. The main difficulty in testing this possibility was to remove the excess of AF after it has reacted with prothrombin so that thrombin formed, if any, from the prothrombin complex by the action of thromboplastin is free to act on fibrinogen. This difficulty was circumvented by adopting the following procedure.

A 2% solution of AF was added dropwise to 0·1 c.c. of a concentrated solution of prothrombin until there was no further formation of precipitate. The granular precipitate formed easily settled down to the bottom of the tube. The mixture was then centrifuged.

(*a*) To an aliquot of the supernatant were added 0·1 c.c. of diluted AC-globulin and 0·2 c.c. venom. After incubating for 5 min. to convert the residual prothrombin, if any, to thrombin, 0·1 c.c. of fibrinogen was added to this mixture and the coagulation time was noted.

(*b*) The precipitate of prothrombin-AF complex was insoluble in water and was therefore washed thrice with distilled water to remove the last traces of free AF.

The washed precipitate was suspended in 0·2 c.c. of distilled water. After adding 0·1 c.c. of dilute Ac-globulin and 0·2 c.c. venom to this suspension, the mixture was incubated for 5 min. After addition of 0·1 c.c. of fibrinogen, the coagulation time was measured.

(*c*) The above experiment was repeated with Bacto 2-stage reagent as a source of thromboplastin.

To 0·1 c.c. of the suspension of the prothrombin-AF complex were added 2·4 c.c. of diluted Ac-globulin. After mixing well, 0·1 c.c. of this suspension was taken and 0·3 c.c. of Bacto 2-stage reagent added. After incubating for 5 min. 0·1 c.c. of fibrinogen was added and the clotting time noted.

TABLE II

Effect of anticoagulant factor on prothrombin activation

Reaction mixture	Clotting time
(*a*) 0·1 c.c. of supernatant obtained after complete precipitation of prothrombin-AF complex + 0·1 c.c. Bacto Ac-globulin (150 times dilution) + 0·2 c.c. venom + 0·1 c.c. fibrinogen	No clotting.
(*b*) 0·2 c.c. of suspension of prothrombin-AF complex + 0·1 c.c. Ac-globulin + 0·2 c.c. venom + 0·1 c.c. fibrinogen	Thick precipitate formation after 1 min. indicating partial inactivation.
(*c*) 0·1 c.c. of suspension of prothrombin-AF complex containing Ac-globulin + 0·3 c.c. Bacto 2-stage reagent + 0·1 c.c. fibrinogen	Clotting in 100 sec.
(*d*) Control experiment using same concentration of prothrombin and other reagents but without AF	Clotting in 7 sec.

The data presented in Table II show that AF does inhibit the first stage of blood clotting, *i.e.*, it interferes with prothrombin activation. It can also be seen that the supernatant obtained after precipitation of prothrombin-AF complex was devoid of prothrombin, because it was incapable of clotting fibrinogen in the presence of thromboplastin, Ac-globulin and calcium ions. This suggests that combination between prothrombin and AF is possibly quantitative.

Iodacetate inactivation

In our earlier publication (Pillai *et al.*, 1955) it was reported that iodacetate treatment caused only a partial inactivation of the anticoagulant factor when plasma was used to test the anticoagulant activity. It was thought that the use of pure coagulation components would throw more light on the mechanism of iodacetate inactivation.

Results obtained with iodacetate-treated AF are summarised in Table III.

TABLE III

Effect of iodacetate-treated anticoagulant factor on the blood coagulation systems

Reaction mixture	Clotting time
1. 0·4 c.c. thrombin + 0·2 c.c. water + 0·1 c.c. cow fibrinogen	10 sec.
2. 0·4 c.c. thrombin + 0·2 c.c. iodacetate-treated AF + 0·1 c.c. cow fibrinogen	11 sec.
3. (*a*) 0·2 c.c. citrated sheep plasma + 0·2 c.c. dilute iodacetate solution (0·07%)+0·4 c.c. thrombin	20 sec.
(*b*) 0·2 c.c. citrated sheep plasma + 0·2 c.c. iodacetate-treated AF + 0·4 c.c. thrombin	No clotting, increase in viscosity of the solution
4. (*a*) 0·1 c.c. of 0·6% fibrinogen (Parke, Davis) + 0·2 c.c. dilute iodacetate solution (0·07%) + 0·4 c.c. thrombin	35 sec.
(*b*) 0·1 c.c. of 0·6% fibrinogen (Parke, Davis) + 0·2 c.c. iodacetate-treated AF + 0·4 c.c. thrombin	40 sec.

Details of the reaction mixtures are given below:

A mixture of 2 c.c. of a 2% solution of AF and 0·8 c.c. of neutralised iodacetate solution (0·25%) was incubated at room temperature for two hours.

1. To 0·1 c.c. of suitably diluted prothrombin containing Ac-globulin was added 0·3 c.c. of Bacto 2-stage reagent and the mixture incubated for 5 minutes at 37° C. to complete the conversion of prothrombin to thrombin.

To this were added 0·2 c.c. of water and 0·1 c.c. of cow fibrinogen and the clotting time was determined.

2. To the thrombin solution prepared as described above, 0·2 c.c. of iodacetate-treated AF and 0·1 c.c. of cow fibrinogen were added and the clotting time measured.

3. In this experiment, citrated sheep plasma was used as a source of fibrinogen.

A mixture of 0·2 c.c. citrated sheep plasma and 0·2 c.c. of iodacetate-treated AF was incubated for 2 min. at 37° C. To this was added 0·4 c.c. of thrombin prepared as indicated above and the clotting time noted.

A control experiment was carried out with the addition of 0·2 c.c. dilute iodacetate instead of the iodacetate-treated AF.

4. To test whether iodacetate-treated AF can combine with fibrinogen and may thus inhibit fibrin formation the following experiment was carried out.

0·1 c.c. of diluted fibrinogen (0·6% solution of a sample of fibrinogen obtained from Messrs. Parke, Davis & Co.) and 0·2 c.c. of iodacetate-treated AF were mixed well and incubated at 37° C. for 2 min. To this solution 0·4 c.c. of thrombin was added and the clotting time measured.

A control was also run with 0·2 c.c. of dilute iodacetate (approx. 0·07%) instead of iodacetate-treated AF.

It was observed that iodacetate treatment completely destroys the antithrombin activity of the anticoagulant factor. Therefore, if thrombin is added to plasma which had been previously incubated with iodacetate-treated AF one would expect that thrombin would be free to act on fibrinogen of the plasma and convert it to fibrin. It was, however, observed that there was no clot formation in presence of iodacetate-treated AF and active thrombin, when citrated plasma was used as a source of fibrinogen. The reason for this anomaly is not clear.

DISCUSSION

As has been shown in the foregoing paragraphs, the anticoagulant factor present in the latex of *Carica papaya* acts as a potent antithrombin inhibiting the second stage of blood coagulation. The factor was equally effective when blood coagulation components prepared from the blood of different species were used (Table I).

That the anticoagulant factor has a definite inhibiting action on the first stage of blood clotting, *viz.*, the activation of prothrombin to give

thrombin, was established by removing the excess anticoagulant from the reaction mixture after incubating it with a concentrated solution of prothrombin (Table II). This was easily accomplished by simple centrifugation since the anticoagulant factor formed an insoluble granular precipitate with prothrombin at high concentrations.

It is probable that the anticoagulant factor has an inhibiting action on thromboplastin, thereby rendering the activation of prothrombin impossible. This could not, however, be ascertained because of the property of AF to form a complex with prothrombin itself.

It was also found that AF forms a precipitate when added to concentrated solutions of thrombin. It can therefore be suggested that this factor forms a soluble complex with thrombin as well as prothrombin at the very low concentrations usually employed in the 2-stage procedure of Ware and Seegers.

Another interesting observation is the retardation of precipitate formation between AF and prothrombin (at high concentrations) when AF is initially incubated with fibrinogen. How exactly fibrinogen alters the property of AF to form a precipitate with prothrombin is not known. The use of conventional physico-chemical procedures like electrophoresis and light-scattering will no doubt help in elucidating the mechanism of this complicated process.

The results obtained with iodacetate-treated AF are as intriguing as they are interesting. It was observed that on treatment with iodacetate the antithrombin activity of AF was completely destroyed. However, when fresh citrated plasma used as a source of fibrinogen was incubated with iodacetate-treated AF, further addition of active thrombin did not result in the formation of fibrin clot (Table III). This suggests that iodacetate-treated AF may form a complex with fibrinogen thus rendering it refractory to the action of thrombin. This could not be confirmed when a dilute solution of purified fibrinogen (Parke, Davis) was incubated with iodacetate-treated AF (Table III).

The only conclusion that could be drawn from these experiments is that iodacetate-treated AF inhibits the formation of a perfect fibrin clot from plasma, probably by forming a complex with fibrinogen in the plasma and that plasma contains a co-factor which promotes this complex formation.

Summary

A detailed investigation has been carried out on the mode of action of the anticoagulant factor present in the latex of *Carica papaya*,

The dual function of the anticoagulant factor, *viz.*, the inhibition of the conversion of prothrombin to thrombin, and the destruction of thrombin activity has been established.

It has been found that the iodacetate treatment destroys the antithrombin activity of the anticoagulant factor. However, the iodacetate-treated anticoagulant factor inhibits the formation of a perfect fibrin clot from plasma, even in the presence of active thrombin, probably through complex formation with the fibrinogen of the plasma. The possibility of the presence of a co-factor in plasma which promotes this complex formation is also envisaged.

ACKNOWLEDGEMENT

Our thanks are due to Prof. W. H. Seegers and Messrs. Parke, Davis & Co., for making available to us a gift sample of pure bovine fibrinogen, to Messrs. Difco Laboratories for their generous gift of the Bacto 2-stage reagent and the Bacto Ac-globulin, and to Messrs. Visking Corporation, Chicago, for a gift sample of seamless Visking casings for dialysis purposes.

REFERENCES

1. Astrup, T. and Darling, S. .. *Acta Physiol. Scand.*, 1942, **4** (1), 1945.
2. Pillai, N. C., Vaidyanathan, C. S. and Giri, K. V. *Proc. Ind. Acad. Sci.*, 1955, **42 B**, 316.
3. Seegers, W. H. .. *Record of Chemical Progress*, 1952 **42 B**, 316.
4. Ware, A. G. and Seegers, W. H. *Am. J. Clin. Path.*, 1949, **19**, 471.

STYLAR END ROT OF THE GUAVA FRUIT (*PSIDIUM GUAYAVA* LINN.)

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Introduction

During the course of the disease survey of the guavas, the author came across a few fruits on the trees in several orchards in the vicinity of Lucknow, showing rot at the stylar end. The disease has, therefore, been termed as 'Stylar end rot'. Survey of these orchards for three consecutive years has revealed that the disease was rare and did not cause much damage to the fruits in the orchard.

The disease was, however, found to be more prevalent in the plucked guavas in storage. Guava fruits showing different stages of the disease were collected fresh from the orchard and some also from the market for investigation of the disease.

A scrutiny of the available literature shows that only a few diseases are recorded on guava fruits. In India only four serious diseases are known, namely, canker caused by *Physalospora psidii* (Uppal, 1936), anthracnose caused by *Pestalozia psidii* (Narsimham, 1938) and *Glœosporium psidii* (Tandon and Agarwal, 1954), and fruit rot due to *Phytophthora parasitica* (1934). Diseases reported from abroad are algal spot (Ruehle, 1941), *Botrytis* rot (Schnellhardt and Heald, 1936), anthracnose due to *Glomerella cingulata* (Palo, 1935) and sun crack (Martyn, 1935). The 'stylar end not' appeared to be a disease hitherto unrecorded and hence detailed investigations were undertaken.

Symptoms

The first visible symptom of the disease is the discolouration in the region lying just below and adjoining the persistent calyx. The discoloured area gradually increases in size, later turning dark brown and the affected area becomes soft (Pl. II, Fig. 4). Along with the discolouration of the epicarp the mesocarp tissue also shows discolouration and the diseased area is marked by being pulpy and light brown in colour in contrast to the bright white colour of the healthy area of the mesocarp (Pl. II, Fig. 7). At an advanced stage due to disorganisation of the inner affected tissues, size of the

fruit shrinks and concentric wrinkles develop on the skin (Pl II, Fig. 5). Finally the whole fruit is affected and is covered with pycnidia.

At all stages of the development of the disease the affected tissue shows abundant fungal hyphæ which are mostly intracellular.

However, even in most advanced stages of the disease, the seeds are not affected.

ISOLATION OF THE CAUSAL ORGANISM

Material and Method.—Diseased fruits fresh from the orchard and in storage were utilised for the isolations. The method employed consisted of surface sterilisation of diseased guavas by swabbing it with 90% alcohol and then allowing the alcohol to evaporate. Later on the skin was removed with a sterile scalpel and a small amount of tissue taken from the periphery of the diseased mesocarp was inoculated in standard synthetic tube slants.

Phomopsis was obtained from all the diseased fruits though sometimes it was associated with some other fungi, *viz.*, *Alternaria*, *Fusarium*, *Aspergillus* and a dark non-sporulating mycelium. However, preliminary pathogenicity tests proved *Phomopsis* to be the causal organism of the disease and later pathogenicity tests were performed with this fungus only.

PATHOGENECITY TESTS

(*a*) *Under laboratory conditions.*—Fresh unripe guavas, 2½ to 4″ in diameter, obtained fresh from the orchards, were utilised. These were surface-sterilised with ·1% mercuric chloride solution and washed with repeated changes of sterile water. They were inoculated by placing a small amount of mycelium from the periphery of five days old monohyphal culture on—

(i) Unwounded epidermis.

(ii) Exposed mesocarp after removal of persistent calyx.

(iii) Shallow pricks on the epidermis made with a sterile needle.

(iv) Deep incision on epidermis made with sterile scalpel.

The inoculated portion was covered with cotton moistened with sterile water and the fruits were incubated at room temperature which varied between 12 to 25° C. in sterile glass chambers. The results of these inoculations are given in Table I.

Phomopsis was found capable of causing stylar end rot in the plucked guava fruits. While injured fruits show 100% infection only 9 out of 30 guavas inoculated on unwounded epidermis produced the disease,

TABLE I

Showing the results of inoculating healthy guava fruits with Phomopsis

Method of inoculation	No. of fruits inoculated	Effect	
		No. of fruits showing rotting	No. of fruits remaining healthy
Shallow pricks	35	35	..
Deep incision	35	35	..
Removing persistent calyx ..	20	20	..
No injury	30	9	21

In the disease produced experimentally after inoculation in the button area the first external symptom discernible on the fourth day is slight discolouration and softening of the tissue just below the persistent calyx. By the sixth day rotting round the inoculated area becomes more pronounced which gradually increases in size and by the tenth day pycnidia of *Phomopsis* make their appearance.

The symptoms of these artificially inoculated fruits follow a similar course as those described for the fruits in the orchard or in storage.

(*b*) *Effect of inoculation of fruits* in vivo.—While successful inoculation of guava fruits was obtained in the laboratory, inoculation was also tried *in vivo*. Mature fruits varying between the size of $2\frac{1}{2}''$ to $3\frac{1}{2}''$ diameter were selected for the experiment and similar methods of inoculation as in the previous case were employed. Table II gives the result of these experiments.

Typical stylar end rot symtoms appeared in fruits showing rotting. The inoculated fruits remaining healthy in the orchard were brought to the laboratory and incubated in sterile chambers at room temperature. One fruit remaining healthy after inoculation with deep scratches showed rotting on the third day after incubation in the laboratory. Out of the six unaffected fruits that were inoculated after shallow pricks, four showed rotting by the third day while two remained healthy even after ten days' incubation in the laboratory.

It will be clear from the foregoing description and Table II that successful infection of the fruits is obtained by *Phomopsis in vivo* also and the affected

TABLE II

Showing the result of inoculating guava fruits with Phomopsis *in vivo*

Method of inoculation	No. of fruits inoculated	No. of fruits remaining on the tree	Effect on the 10th day	
			No. of fruits showing rotting	No. of fruits remaining healthy
Shallow pricks	10	10	4	6
Deep scratches	10	8	7	1
Removing persistent calyx ..	5	5	5	..
No injury	5	4	..	4

fruits develop typical stylar end rot. While infection was readily obtained by deep injury and removal of the persistent calyx, the injury caused by shallow pricks was not so effective.

The fungus was also capable of producing rot in healthy fruits when inoculated in regions other than stylar end.

(*c*) *Effect of the disease on the viability of the seeds.*—50 guava seeds collected from fruits showing advanced stage of rotting were utilised to study the effect of rotting on their viability. The seeds were washed with water and then were surface-sterilised with ·1% mercuric chloride solution. These were later washed with repeated changes of sterile water and were sown in saw dust contained in earthen pans.

By the fourth week, 43 seedlings had emerged out showing thereby that the rotting of the fruits had no effect on the viability of these seeds. However, 7 seeds did not germinate and it is not uncommon to find certain percentage of seeds even in healthy fruits that do not germinate.

MORPHOLOGY OF THE FUNGUS

The fungus produces dark carbonaceous pycnidia on rotting fruits and also in culture. Under moist conditions, masses of spores ooze out from these pycnidia through the ostiole. In standard synthetic media, the mycelial growth is usually chalky white forming compact mats.

Pycnidia which are ovoid and thick-walled are formed readily in older cultures, mainly towards the periphery of the medium, and are also found

on decaying fruits. They measure between 140 to 400 μ in diameter with an average of 225 μ.

The characteristic conidia and slytospores are present though their relative abundance is variable. Their characters are as follows:—

Conidia.—Ovate to elongate, hyaline varying between 5 to 9 μ by 2·5 to 4 μ with an average of 6·8 by 3·1 μ.

Stylospores.—Long, slender, curved and hooked varying between 16 to 32 μ by ·8 to 1·5 μ with an average of 26 μ by 1·2 μ.

The temperature relation of the fungus was studied in culture and it was found that it had a minimum temperature for growth near about 10° C. with an optimum of about 25° C. and maximum of 35° C.

So far no perfect stage of the fungus has been found either in nature or in culture medium.

On the basis of the abovementioned characters the fungus has been identified as *Phomopsis.* The specific identification has not been established so far.

Discussion and Conclusion

The stylar end rot of guava has been found to be due to a species of *Phomopsis.* The role of *Phomopsis* in causing fruit rots is well known. Fawcett (1911 and 1912) attributed stem end rot of citrus fruits due to a species of *Phomopsis* (*P. citri*). Stevens and Piper (1941) describe stem end rot of Avacado in Florida due to a species of *Phomopsis,* while another stem end rot of potato is known to be caused due to *Phomopsis tuberivora* (Foster and Macleod, 1932). Apart from causing stem end rots in various fruits, species of *Phomopsis* are known to cause other type of fruit rots also. Baker (1939) reports a soft rot of tomato fruit caused by a species of *Phomopsis,* while Wollenweber and Hochapfel (1936) describe *Phomopsis enterolenca* causing rot of pear and *Phomopsis ambigua* causing rot of apple, pear and quince fruits. Hildebrand (1947) has referred to *Phomopsis citri* attacking immature fruits of citrus. Walter (1950) has described mummification of tomato fruits being caused due to a species of *Phomopsis.*

Typically the guava fungus causes stylar end rot but it was also found capable of producing fruit rot in general. Some infection was obtained through unwounded surface also but wounding favoured the infection and so also the entry of the pathogen. This is in agreement with the observation of Fawcett (1936) for stem end rot of citrus fruits caused by *Phomopsis citri* and Foster and Macleod (1932) for stem end rot of potato. Injury caused by breaking the persistent calyx seemed to be an effective way of causing

injury as a high percentage of infection was obtained in this way. Probably this explains why the disease starts from the stylar end as it is very likely that the persistent calyx is broken during the picking of fruits or in transit exposing it to fungus infection.

The mycelium of the fungus is slow growing, having an optimum temperature of about 25° C. with about 10° C. and 35° C. as minimum and maximum. Similar temperature relationships are known for other species of the fungus, *viz.*, Fawcett (1936) reports for *Phomopsis citri* to have an optimum of about 23° C. while Foster and Macleod (1932) give an optimum between 20–25° C. for *Phomopsis tuberivora*. This optimum for the growth of the fungus shows why guava fruits are susceptible to this rot even during winter months when fruit rots due to other tropical fungi like *Aspsergillus niger* (Savastano and Fawcett, 1929) are very rare.

SUMMARY

The paper deals with investigations on the stylar end rot of guava fruits. Fruits in the orchard as well as in storage suffer with this disease, which is, however, of rare occurrence. The symptoms of the disease have been described in detail.

A species of *Phomopsis* was constantly associated with this rot and later pathogenicity tests proved this fungus to be the causal organism.

While some infection was obtained through uninjured surface, injury favoured infection. Apart from stylar end rot the fungus was able to cause fruit rot in general.

The morphology of the fungus has been described and its temperature relationship has been studied. The fungus had a minimum temperature for growth near 10° C., an optimum near 25° C. and a maximum of about 35° C.

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LITERATURE CITED

1. Brown, N. A. .. "Tumors on Elm and Maple trees," *Phytopathology*, 1941, **31**, 541–48.
*2. Baker, R. E. D. .. "Notes on diseases and fruit rots of tomatoes in the British West Indies," *Trop. Agriculture Trin.*, 1939, **16** (11), 252–57 (*Abst. Rev. Appl. Myc.*, 1940, **19**, 170).
3. Chardon, C. E., Miller, J. H. and Muller, A. S. "*Phyllachora tropicalis* on guava," *Mycologia*, 1942, **32** (2), 172–204.
4. Das Gupta, S. N. and Rai, J. N. "Witl diseases of Guava," *Curr. Sci.*, 1947, **16**, 257–58.

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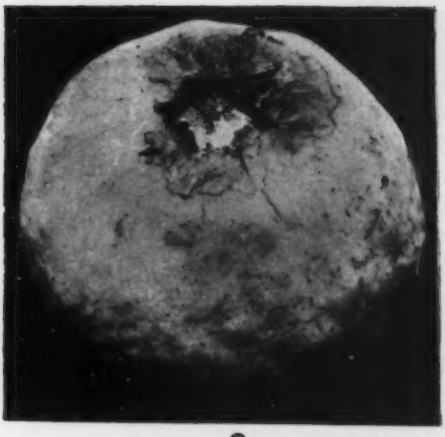

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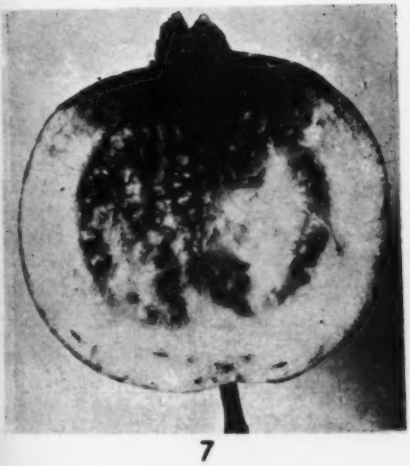

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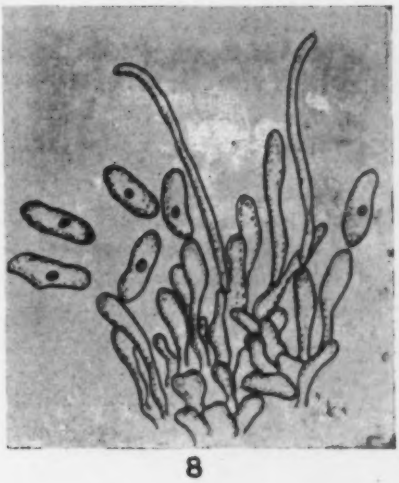

8

*5. Fawcett, H. S. .. "Stem end rot of Citrus fruit (*Phomopsis* sp.)," *Florida Agr. Expt. Sta. Bull.*, 1911, **107**, 23.

6. ———— .. "The cause of Stem end rot of Citrus fruits (*Phomopsis citri* n. sp.)," *Phytopathology*, 1912, **2**, 109–13.

7. ———— .. *Citrus Diseases and Their Control.* McGraw-Hill Publications, 1936, p. 440–55.

*8. ———— .. "Citrus diseases of Florida and Cuba compared with those of California," *California Agr. Exp. Sta. Bul.*, 1915, **20**, 261.

9. Hildebrand, E. M. .. "Stem end rot fungi attacking immature Citrus fruits," *Phytopathology*, 1947, **37**, 433.

*10. Martyn, E. B. .. "Sun crack of guava," *Div. Rept. Dept. Agric. Brit. Guiana*, 1934, p. 105–08.

*11. Macleod, H. S. .. "A new stem end rot of potato," *Canadian Journal of Research*, 1932, p. 520–23 (*Abst. Rev. Appl. Myc.*, 1933, **12**, 241).

*12. Narsimham, M. J. .. "*Pestalozia psidii* on guava," *Adm. Rept. Agric. Dept. Mysore*, 1936–37, pp. 169–73.

*13. Palo, M. A. .. "Notes on mottle leaf and anthracnose fruit spot of *Citrus* in Bukiduon," *Philipp. J. Agric.*, 1935, **6**, 497–500.

14. Rai, J. N. .. "Internal breakdown of guava fruit (*Psidium guayava* L.)," *Curr. Sci.*, 1948, **17**, 273–74.

15. Ruehle, G. D. .. "Algal leaf and fruit spot of guava," *Phytopathology*, 1941, **31**, (1), 195–96.

16. Savastano, G. and Fawcett, H. S. "A study of decay in Citrus fruits produced by inoculation with known mixtures of fungi at different constant temperatures," *Journ. Agr. Res.*, 1923, **39**, 163–98.

17. Schnellhardt, O. and Heald, F. D. "Pathogenicity tests with *Botrytis* species," *Phytopathology*, 1936, **26**, 786–94.

18. Seth, L. N. .. "Studies in the genera *Cytosporina*, *Phomopsis* and *Diaporthe*, V," *Ann. Bot.*, 1934, **189**, 69–107.

19. Tandon, R. N. and Agarwala, R. K. "Pathological studies of *Glæosporium psidii* causing die back of guavas," *Proc. Ind. Acad. Sci.*, 1955, **40**, 102–09.

20. Uppal, B. N. .. "*Physalospora psidii* on Guava, a serious disease of guava in Bombay," *Int. Bull. Pl. Bot.*, 1936, **10** (5), 99.

21. Walter, H. J. .. "A *Phomopsis* disease of Tomatoes in Hawaii," *Phytopathology*, 1950, **40**, 966.

*22. Wollenweber, H. W. and Hochapfel, H. "Beitrage Zur Kenntnis parasitarer and sprophytischer Pilze, 1. Phomopsis, Dendrophoma, Phoma und Ascochyta und ihre Bezichung zur Frachtfaule," *Z. Parasitenk*, 1936 **8** (5), 561–605 (*Abst. Rev. Appl. Myc.*, 1937, **16**, 105–06).

23. *Bombay Agricultural Department Bulletin No.* 176, 1934.

Explanation of Plate

Figs. 1–8. Figs. 1–3. Fruits showing different stages of the disease. Fig. 4. A fruit showing fairly advanced stage of stylar end rot with characteristic discolouration of the affected portion. Fig. 5. A fruit showing advanced stage of the disease when nearly half of the fruit has been affected. Wrinkles and pycnidia of *Phomopsis* have appeared on the surface of the fruit. Fig. 6. Seedless guava fruit, a rare variety showing the disease in the orchard. Fig. 7. A diseased fruit split open to show the discoloured affected area in contrast to the white healthy portion. Fig. 8. C.L. sketch thorgh a portion of the pycnidium on the fruit showing the stylospores and conidia, ×1,180.

* Literature not consulted in original.

THE ACTION OF LEAD ON UNSTRIATED MUSCLE AND BLOOD VESSELS AND ITS RELATION TO HYPERTENSION

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Chronic hypertension and arteriosclerosis may be associated with chronic lead poisoning, but it does not appear to be more common in lead workers than in others (see Sollmann, 1948, for references); nor they can be induced by long continued lead administration in rabbits and dogs. The blood pressure is not affected by lead poisoning, except during colic attacks when it rises. Intestinal spasm may be produced by intravenous injection of lead acetate and in excised intestines. It is by no means clear how it produces colic, although it is known that the disturbance is due to spasm of the intestine. Though some relief may be obtained in man and experimental animals by atropine, there seems to be no known agent which relieves lead colic. Certain authors (Aub *et al.*, 1925) assume that the action is due to the effect of lead on the envelope or membrane of smooth muscle cells, others (Grünberg, 1928), believe that it acts on the ganglion cells in the local plexus which control intestinal action.

Spasmodic contracture of the retinal vessels is known to occur in lead poisoning. The excised or perfused arteries are constricted by lead salts (Sollmann, 1948). This action is probably due to the action of lead on the excitatory system of smooth muscle.

It is universally accepted that the metabolism of lead and calcium are closely linked, so that the factor which would favour deposition of calcium in the media of the blood vessels, would also favour the deposition of lead, which might then act on the contractile mechanism of the smooth muscle of the blood vessels, and cause tonic spasm in this manner. The calcium content of the arteries increases in the aged, so that as the arteries have a special affinity for calcium, they may have a similar one for lead, though no analyses of the lead content of arteries are available. If they do, then lead will be especially present in increased concentration in the media of the arteries, and so produce a direct effect on the contractile mechanism of their smooth muscle. In the present research, therefore, the action of lead on the contractile mechanism of smooth muscle has been studied, in order to elucidate its possible role in hypertension.

Experimental

The action of lead on the excitatory mechanism of unstriated muscle was studied by testing the effect of lead chloride on transverse muscle of the excised stomach of the dog and the frog, *Rana tigrina*. For testing the action on the contractile mechanism, transverse pieces of frog's stomach muscle were used, the action was tested on dying muscles the pieces of muscle being immersed in lead solutions for 24 hours (Singh and Singh, 1949, 1950), and on the heat killed frog's stomach muscle (Singh and Singh, 1954 *a*, *b*, *c*; 1955 *a*).

For studying the effect on the contractile mechanism of the smooth muscle of the blood vessels, the dog's hind limbs were perfused and the rate of flow recorded before and after immersion for 24 hours (Singh and Singh, 1955 *b*, *c*, *d*).

Direct microscopic observations on the arterioles of the guinea pig's mesentery were made before and after immersion for 24 hours.

Results

Action of lead on the excitatory mechanism.—Saline which was saturated with lead chloride, was used. Other lead salts, such as acetate, nitrate, though more soluble, were not used as the anions have a stimulating effect on unstriated muscle. Lead chloride does not produce any contraction of dog's and frog's stomach muscle. In dog's stomach muscle, it produces only relaxation.

Arterioles of the dog's hind limb are constricted, as shown by immediate retardation of flow in concentrations of lead chloride from 1 in 10^5 to 1 in 10^4. The arterioles of the guinea pig's mesentery are also constricted.

Lead therefore acts on the excitatory mechanism of smooth muscle. Lead increases the excitability to alternating current and potassium in small concentrations, and in higher concentrations, it increases the excitability to alternating current and decreases that to potassium (Singh and Singh, 1946).

Action of lead on the contractile mechanism.—The contractile mechanism of unstriated muscle is affected by lead. In dying muscles, immersed for 24 hours, lead chloride causes contraction, in concentrations up to 1 in 10^5 (Table I). Heat killed muscles are contracted by concentrations up to 1 in 1000 (Table II). Dying arterioles are also affected similarly; concentrations up to 1 in 10^5 causes them to contract, as shown by perfusing dog's hind limbs (Fig. 1).

TABLE I

Frog's stomach muscle. Contraction produced by lead chloride in dying muscle, immersed for 24 hours

No. of Experiment	Contraction produced by lead chloride, saturated saline: Per cent. of initial length — Effect of lead	Control	No. of Experiment	Per cent. of initial length — Effect of lead	Control
1	83	91	7	90	96
2	78	90	8	90	85
3	71	100	9	62	90
4	80	115	10	74	80
5	90	110	11	74	90
6	71	98	12	75	85
	Effect of lead, 1 in 10^4				
1	90	96	4	74	80
2	90	85	5	74	90
3	62	90	6	75	85
	Effect of lead, 1 in 10^5				
1	81	90	4	85	100
2	80	90	5	75	100
3	79	110	6	70	90

TABLE II

Frog's stomach muscle, killed by heating to 50° C. for 10 minutes. Contraction produced by lead chloride, immersed for 24 hours

No. of Experiment	Contraction produced by lead chloride, saturated saline: Per cent. of initial length — Effect of lead	Control	No. of Experiment	Per cent. of initial length — Effect of lead	Control
1	93	100	4	90	102
2	90	105	5	90	108
3	95	105	6	98	108
	Effect of lead 1 in 1000				
1	100	104	4	103	106
2	95	100	5	90	98
3	104	110	6	95	105

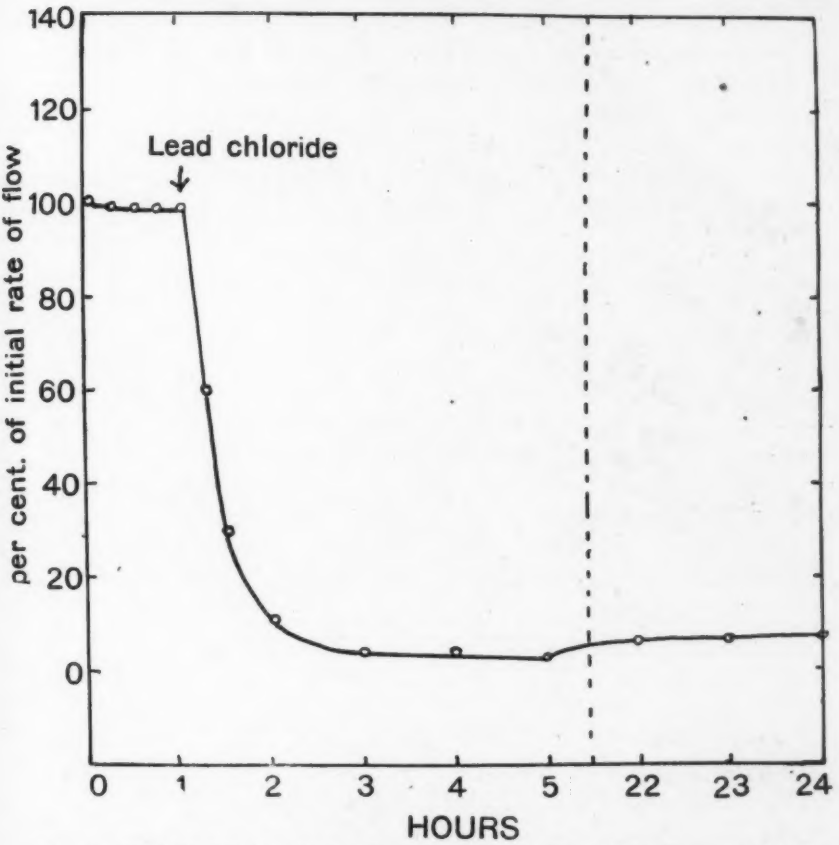

FIG. 1. Dog's hind limbs. At first they were perfused with unbuffered mammalian saline, and then with saline saturated with lead chloride.

DISCUSSION

Lead may affect the smooth muscle in the body by acting on the excitatory or the contractile system. In the latter case, the spasm will not be amenable to drugs. If the metabolism of lead follows that of calcium, then lead is likely to be concentrated in the arteries, just as calcium, and thus persistent hypertension may result. It is however doubtful whether such action of lead occurs in the body.

SUMMARY

1. The action of lead on the excitatory and contractile mechanism of smooth muscle is described. The latter has been determined by testing the effect of lead on dying and heat killed muscles.

2. Lead causes contraction of smooth muscle and arterioles by direct action on the contractile mechanism. This action may be of significance in producing contraction of smooth muscle in the body and in producing hypertension.

REFERENCES

Aub, J. C., Fairhall, L. T., Minot, A. S. and Reznikoff, P. .. *Medicine*, 1925, **4**, 1. Quoted from W. T. Salter, *A Text Book of Pharmacology*, W. B. Saunders, London, 1953, p. 983.

Grünberg, F. W. .. *Ztschr. f. d. ges. exper. Med.*, 1928, **62**, 649. Quoted from Salter.

Singh, S. I. and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1946, **23**, 301.

———— .. *Ibid.*, 1949, **30**, 270.

———— .. *Ibid.*, 1950, **32**, 12.

———— .. *Ibid.*, 1954 *b*, **40**, 125.

———— .. *Ibid.*, 1954 *c*, **40**, 145.

———— .. *Ibid.*, 1955 *a*, **41**, 183.

———— .. *Ibid.*, 1955 *b*, **42**.

———— .. *Ibid.*, 1955 *c*, **42**.

———— .. *Ibid.*, 1955 *d*, **42**.

———— .. *Curr. Sci.*, 1954 *a*, **23**, 126.

Sollmann, T. .. *A Manual of Pharmacology*, 7th edition, 1948, p. 989, W. B. Saunders, London.

TAXONOMY OF THREE SPECIES OF *STRIGA* PARASITIC ON SUGARCANE

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In Bihar, sugarcane is parasitised by two species of *Striga* namely *S. lutea* and *S. euphrasioides*, the former being more common and destructive than the latter. They have been differentiated by Duthie (1911), Haines (1922) and Santapu (1952) on the basis of number of calyx-ribs, it being 10 for *S. lutea* and 15 for *S. euphrasioides*. Pennell (1943) placed all the species into two broad groups (1) 4–5 ribs in calyx and (2) 10–15 ribs in calyx and differentiated between these two species on the basis of colour of corolla and branching of stem. According to him, *S. lutea* and *S. euphrasioides* have respectively yellow and white corolla and the stem of the former is less branched than that of the latter. In Bihar both of the species have white flowers and *S. lutea* is much more profusely branched than *S. euphrasioides*. *S. densiflora* is separated by Duthie (1911) and Santapu (1952) from *S. gesneroides* and *S. orobanchioides* by its non-parasitic habit although Kumar and Solomon (1941) and Sharma *et al.* (1954) found it to grow respectively on 24 and 8 plants either in nature or under laboratory conditions. With a view to broadbase the diagnosis of these species on a sounder footing a detailed study of these three species was made (Table I).

Since the number of calyx-ribs was regarded as the basic diagnostic feature by all the previous workers, 300 flowers of each species were examined for this character, which was found to show great variation in at least two species (Table II). There were 10 to 15 ribs in *S. lutea*, 13 being most frequent (50%), and 14 to 18 in *S. euphrasioides*, with 16 having the highest frequency (69·6%).

S. densiflora in which 94·4% of flowers had 5 calyx-ribs, showed a far greater consistency so far as this character was concerned.

On the basis of these characters the following key of identification for these three species is suggested.

TABLE I

Detailed description of three species of Striga

Characters		*S. densiflora*	*S. lutea*	*S. euphrasioides*
1. Stem (*a*) Length in cm.		15–30 cm.	15–30 cm.	35–50 cm.*
(*b*) Branching		5–12 cm. above soil surface. Main shoot distinct.	Profuse branching (upto 72 branches); emerging from below soil surface or just above it. Main shoot not distinct.	Branches usually 4–8; beginning at 5–12 cm. above ground level. Main shoot distinct.
(*c*) Below soil surface		Scaly leaves present, stem bluish in colour deepening on drying.	Scaly leaves present; Stem of dull brown colour.	Scaly leaves absent; Stem bluish like those of *S. densiflora*.
2. Length of leaf in cm.	Max.	2·70	3·00	6·30
	Min.	0·50	1·20	1·70
	Avr.	1·59	2·20	4·29
3. Flower—				
(*a*) Bractiole		More or less equal or longer than calyx.	Shorter than calyx.	Shorter than calyx.
(*b*) Calyx shape		Tooth lanceolate; marginal hairs spreading (Fig. 4 *a*).	Tooth linear. Secondary ribs terminating in sinus; marginal hairs spreading (Fig. 4 *c*).	Tooth linear, secondary ribs continued to the extremity of the tooth; marginal hairs curved upwards (Fig. 4 *b*).
(*c*) Size in mm.	Max.	8·5	8·5	13·0
	Min.	4·0	4·0	7·0
	Avr.	6·0	8·0	10·7

	Avr.	6·0	8·0	10·7
4. Roots		Poorly developed.	Poorly developed.	Well developed.
5. Fruit Length in mm.	Max.	5·0	4·6	8·0
	Min.	3·0	2·6	5·0
	Avr.	3·9	3·6	6·4
Breadth in mm.	Max.	2·6	2·1	3·6
	Min.	1·3	1·0	2·4
	Avr.	2·0	1·6	2·9
6. Seed—				
(*a*) Colour		Darkish brown.	Coffee brown.	Jet Black.
(*b*) Germination without host contact ..		Nil	Nil	80%
(*c*) Length in μ	Max.	565	411	743
	Min.	171	197	436
	Avr.	341	310	569
Breadth μ	Max.	265	254	340
	Min.	124	151	206
	Avr.	181	204	289
7. Estimated number of plants per acre in Bihar ..		..	Upto 6,00,000	Upto 2,000

* It may attain a height upto 115 cm. inches (Khanna, 1946).

TABLE II

Number of calyx-ribs in three species of Striga

Species of *Striga*	No. of calyx ribs	I lot		II lot		III lot		IV lot		V lot		VI lot		Total	
		*F.	P.	F.	P.	F.	P.	F.	P.	F.	P.	F.	P.	F.	P.
S. lutea	10	1	2	2	4	2	4	2	4	3	6	5	10	15	5·0
	11	4	8	4	8	2	4	5	10	4	8	9	18	28	9·3
	12	9	10	12	24	11	22	14	28	10	20	14	28	70	23·3
	13	32	64	23	46	25	50	25	50	27	54	18	36	150	50·0
	14	4	8	9	18	9	18	3	6	6	12	9	18	35	11·7
	15	..	..	..	..	1	2	1	2	..	..	..	..	2	0·7
S. euphrasioides	14	7	14	2	4	1	2	1	2	1	2	1	2	13	4·3
	15	12	24	7	14	5	10	13	26	11	22	8	16	56	18·7
	16	29	58	38	76	41	82	33	66	35	70	33	66	209	69·6
	17	2	4	3	6	3	6	2	4	3	6	7	14	20	6·7
	18	..	..	..	..	..	..	1	2	..	..	1	2	2	0·7
S. densiflora	4	2	4	1	2	2	4	2	4	3	6	..	..	10	3·3
	5	47	94	48	96	47	94	46	92	46	92	49	98	283	94·4
	6	11	2	1	2	1	2	2	4	1	2	1	2	7	2·3

* F. = Frequency. P. = Per cent.

* F. = Frequency. P. = Per cent.

FIG. 3

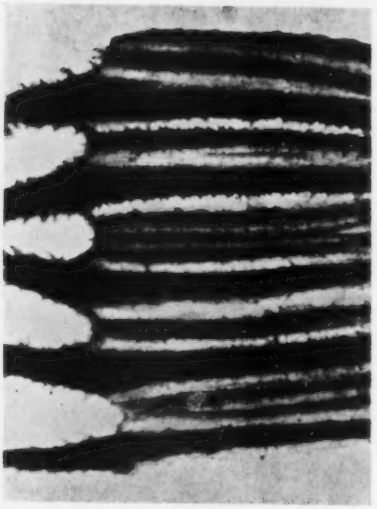

FIG. 2

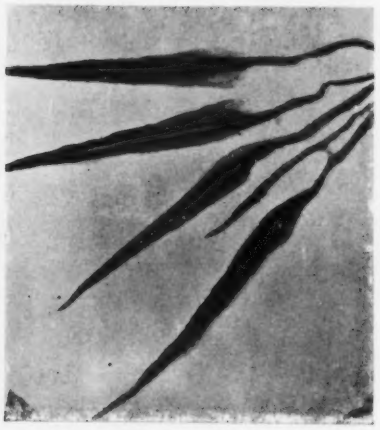

FIG. 1

FIG. 4 (b)

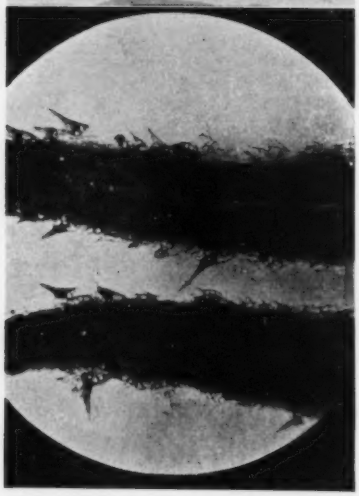

FIG. 4 (a)

Key of identification for 3 species of Striga

A	Calyx tooth lanceolate: ribs 4–6 (Fig. 1)	1. *S. densiflora.*
AA	Calyx tooth linear	
B	Ribs 10–15 mostly 12 or 13; capsule 2·5 to 4·5 mm. long. Secondary ribs ending in sinus (Fig. 2)	2. *S. lutea.*
BB	Ribs 14–18 mostly 15–16; capsule 5·0 to 8·0 mm. long. Secondary ribs continued into the extremity of calyx tooth (Fig. 3)	3. *S. euphrasioides.*

Acknowledgement

Authors are indebted to Sri. K. L. Khanna, Director, Sugarcane Research and Development, Bihar, for necessary facilities and keen interest in the progress of this work and Sri. S. K. Mukerjee, Indian Botanic Gardens, Sibpore, Calcutta, for the identification of specimens and to Prof. L. S. S. Kumar, Economic Botanist to the Government of Bombay, for the supply of seed of *Striga densiflora.*

References

1. Duthie, J. F. .. *Flora of the Upper Gangetic Plain,* 1911, **2**, 155.
2. Haines, H. H. .. *Botany of Bihar,* 1922, Part IV, 640–61.
3. Khanna, K. L. .. *Ann. Rep. Cent. Sug. Res. Sta.,* Pusa, Bihar, 1945–46, 68.
4. Kumar, L. S. S. and Solomon, S. "A list of phanerogamic root parasites attacking economic crops in India," *Proc. Ind. Acad. Sci.,* 1941, **13**, 151–56.
5. Pennell, F. W. .. "The *Scrophulariaceæ* of the Western Himalayas," *The Acad. Nat. Sci., Philadelphia, Monograph* No. 5, 1943.
6. Santapu, H. .. "*Scrophulariaceæ* of the Bombay Presidency," *J. of Bombay Nat. Hist. Soc.,* 1952, **49**, 417–38.
7. Sharma, S. L., Rao, D. and Trivedi, K. N. "New hosts of three species of *Striga,*" *Proc. II Bin. Conf. of Sug. Workers in Ind. Union,* Part I, 1954, p. 75 (*Abstract*).

Explanation of Plate

Figs. 1–4. Fig. 1. Calyx of *Striga densiflora* with 5 ribs. Fig. 2. Calyx of *S. lutea* showing 14 ribs. Fig. 3. Calyx of *S. euphrasioides* showing 18 ribs. Fig. 4. (*a*) Marginal hairs in *S. densiflora*; spreading. (*b*) Marginal hairs in *S. euphrasioides*; curved upwards. (*c*) Marginal hairs in *S. lutea*; spreading.

A CONTRIBUTION TO THE LIFE-HISTORY OF *OROXYLUM INDICUM* VENT

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(Communicated by Dr. I. Banerji, F.A.Sc.)

Introduction

Oroxylum indicum Vent, a plant belonging to the family Bignoniaceæ, is grown as an ornamental plant in many gardens. It is a tree with large, beautiful bilabiate flowers and long sword-shaped fruits.

The earlier literature on the embryology of the Bignoniaceæ has been reviewed by Schnarf (1931). Since then the following important contributions have been made. Mauritzon (1935) has described the formation of the embryo-sac and endosperm in six genera and has recognised two main types of endosperm development. Soueges (1940) for the first time, studied in detail the development of the embryo in *Catalapa kæmpferi*. Swamy (1941) has given an account of the embryology of *Bignonia megapotamica* and later Govindu (1950) described in detail the embryology of *Jacaranda mimosæfolia*, *Parmentiera cerifera*, *Tecoma stans* and *Kigelia pinnata*.

The literature on the cytology of the family is comparatively meagre. The earlier cytological investigations as reported by Wang (1940), Raghavan and Venkatasubban (1940), Venkatasubban (1945) and Darlington and Janaki Ammal (1945) refer mainly to the chromosome numbers of the various genera of the family.

The present work gives an account of the cytology and embryology of *Oroxylum indicum*. The development of the wing on the seed has also been studied.

Material and Methods

The material used in the present investigation was obtained from a plant growing in the University College campus.

For cytological studies, temporary and permanent preparations of anthers and root-tips were made. Meiotic division was frequent between 9 A.M. and 11 A.M. For permanent preparations, anthers were treated in equal proportions of ·002 M Oxyquinoline and Nawaschin's fluids (Sharma and Ghosh, 1951) at 18° C. for two hours and then kept at room temperature

for overnight. Temporary smears of anthers in acetocarmine were not satisfactory.

Temporary mounts of root-tips were treated in a mixture of saturated solution of paradichlorobenzene and water in the proportion of 1 : 4 with a drop of formalin for 45 minutes at room temperature; heated in a mixture of 2% aceto-orcein and N.HCl (9 : 1) and squashed in 1% aceto-orcin. Very clear metaphase plates were obtained by this method. For permanent mounts, fixation of root-tips in a mixture of equal amounts of 1% platinic chloride and 10% formalin gave satisfactory results.

For embryological studies, ovaries in different stages of development were fixed in Nawaschin's fluids. Dehydration, clearing and embedding were done in the usual way. Sections were cut 8–16 μ thick depending on the stage required for study.

Newton's crystal-violet-iodine and Heidenhain's iron-alum hæmatoxylin were used for staining.

Pollen grains were stained and mounted in methyl-green-glycerine jelly for study.

For studying the nature of the cells composing the wing of the seeds, these were macerated in 5% chromic acid and also in saturated solution of caustic potash.

Observations

The Somatic Chromosomes

The diploid number of chromosomes as determined from root-tip cells was found to be 28 (Fig. 1). On the basis of relative length, the chromosomes could be arranged under three categories:—

(i) One pair of long chromosomes 3·67 μ in length.

(ii) Eight pairs of medium-sized chromosomes varying in length from 2·45 μ to 1·53 μ.

(iii) Five pairs of short chromosomes varying in length from 1·42 μ to 1·12 μ.

An idiogramatic representation of the chromosomes reveals the following types: (Fig. 2)

Type A.—One pair of long chromosomes with submedian primary constriction and secondary constriction on the shorter arm.

Type B.—One pair of medium-sized chromosomes with satellites. Primary constrictions are median.

Type C.—One pair of medium-sized chromosomes with satellites and sub-median primary constriction. Satellites are situated on the shorter arm.

Type D.—One pair of medium-sized chromosomes with submedian primary constriction and secondary constriction on the shorter arm.

Type E.—One pair of medium-sized chromosomes with sub-median primary constriction.

Type F.—One pair of medium-sized chromosomes with satellites and sub-median primary constriction. Secondary constriction is on the longer arm.

Type G.—One pair of medium-sized chromosomes with sub-median primary constriction.

Type H.—One pair of medium-sized chromosomes with median primary constriction.

Type I.—One pair of medium-sized chromosomes with sub-median primary constriction.

Types J and M.—Two pairs of short chromosomes with median primary constrictions.

Types K, L, and N.—Three pairs of short chromosomes with sub-median constrictions.

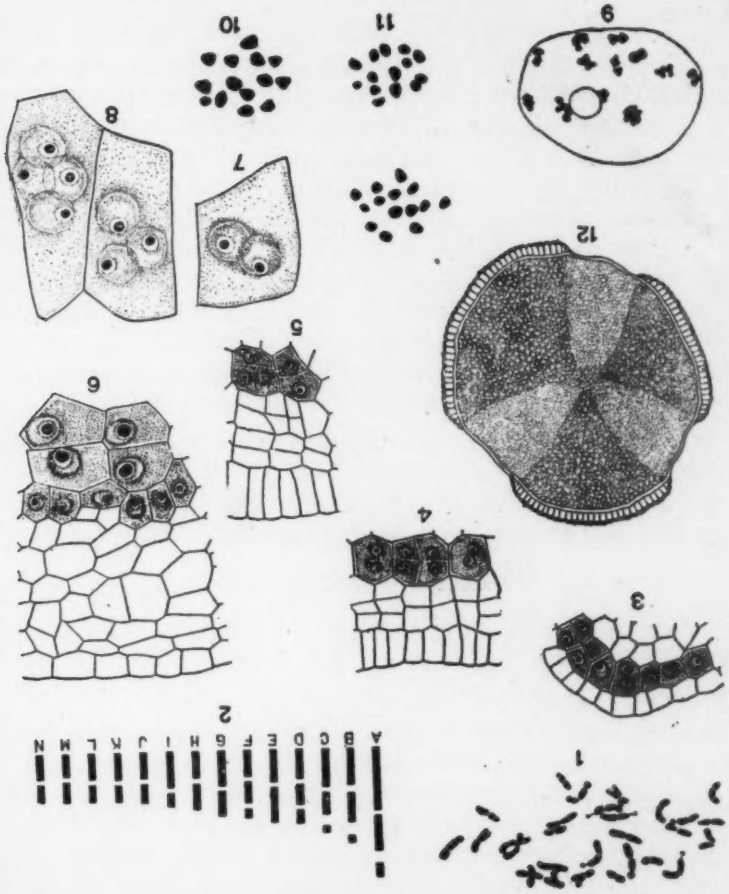
MEIOSIS AND THE POLLEN GRAINS

In the hypodermal layer a row of single-layered hypodermal archesporial cells becomes distinguishable during the lobation of the anther (Fig. 3). The archesporium is recognisable by the larger size, denser cytoplasm and more prominent nuclei of its cells. It divides periclinally to produce an outer layer of parietal cells and an inner of sporogenous cells (Fig. 4). By further divisions of the parietal cells, at least nine layers of cells are produced in the mature anther of which the innermost forms a secretory tapetum (Figs. 5 and 6). The outer layer forms the conspicuous hypodermal endothecium which later becomes multi-layered below and show the characteristic endothecial bands. The epidermal cells show prominent radial elongation and conspicuous cutinization on the outer surfaces.

Before the differentiation of the microspore-mother-cells, the sporogenous tissue divides only once producing two layers (Figs. 4, 5 and 6). This division of the sporogenous layer takes place before the maturity of the tapetum (Figs. 4 and 5). The tapetal cells which are uninucleate at the initial stages, become binucleate (Figs. 6 and 7). Some of these cells later become

ERRATUM

In Vol. XLIII, No. 1, on p. 75 the block of Figs. 1 to 12 is inverted.



TEXT-FIGS. 1–12. Fig. 1. Somatic metaphase, ×2,450. Fig. 2. Idiogram of the somatic chromosomes, ×4,900. Figs. 3–6. Differentiation of parietal and sporogenous tissue in the anther initial, ×500. Fig. 3. Hypodermal archesporium in the anther, ×500. Figs. 4–5. Division of the parietal and the sporogenous tissues. ×500. Fig. 6. Differentiation of the tapetal cells, ×500. Fig. 7. Binucleate tapetal cells, ×1,050. Fig. 8. Tri- and Quadrinucleate tapetal cell, ×1,050. Fig. 9. Diakinesis in a P.M.C., ×1,050. Fig. 10. Meiotic metaphase I, ×2,450. Fig. 11. Meiotic metaphase II, ×2,450. Fig. 12. A pollen grain, ×750.

quadrinucleate (Fig. 8). The tapetal cells begin to degenerate after the formation of the pollen grains.

Meiosis does not show any unusual feature. At diakinesis fourteen bivalents are seen, of which three are attached to the nucleolus (Fig. 9).

The bivalents are somewhat irregular in form but show size differences and lie mostly towards the periphery of the nucleus. The nucleolus shows progressive reduction in size and disappears along with the disappearance of the nuclear membrane. During metaphase I, the chromosomes are regularly arranged on the equatorial plate of the bipolar spindle and become much condensed. A polar view of an equatorial plate shows fourteen bivalents (Fig. 10). The anaphasic separation of the chromosomes appears to be regular. On the completion of the first division, the two daughter nuclei are organised with distinct nuclear membranes and the chromosomes pass through an interkinetic stage. Second division is of the simultaneous type, fourteen chromosomes appear like dots in polar view at metaphase II (Fig. 11). On the completion of the second division, four daughter nuclei are formed arranged in a tetrahedral manner. Cytokinesis takes place by the process of furrowing.

The mature pollen grains are tricolpate, spheroidal-prolate, length of the longest axis varies from 70 μ–80 μ; reticulate, sexine is entire, annular and thicker than the nexine (Fig. 12). The pollen grains are binucleate, the vegetative nucleus being much bigger in size than the generative nucleus.

DEVELOPMENT OF THE OVULE

As is characteristic of the Bignoniaceæ, the ovary is distinctly superior, subsessile, bicarpellary and bilocular with a single style and a bilobed flattened stigma. A thick, annular disc occurs at the base of the ovary.

The ovules are unitegmic, tenuinucellate and mostly anatropous. A few hemianatropous ovules have also been noted. The ovules are borne on the two slightly projecting placentas. The placentas are parietal in the beginning (Fig. 13) but very soon become axile due to the later development of a sterile, central bridge connecting the two fertile, massive projections (Fig. 14).

The ovule at first arises as an uniform mass of a papillate protrusion of the placenta and curves inwards before the differentiation of the archesporium and the integument. The direction of curvature of the ovules is opposite to each other on the two sides of the sterile partition wall (Fig. 14).

A hypodermal archesporial cell differentiates before the integument is developed (Fig. 15). The integument arises from the base of the nucellus (Fig. 16) when the ovule has rotated about ninety degrees. The integument at its inception is composed of only two layers of cells and extends to about half the length of the nucellus. The cells composing the integument divide rapidly and the tip is soon seen to be composed of four layers of cells which

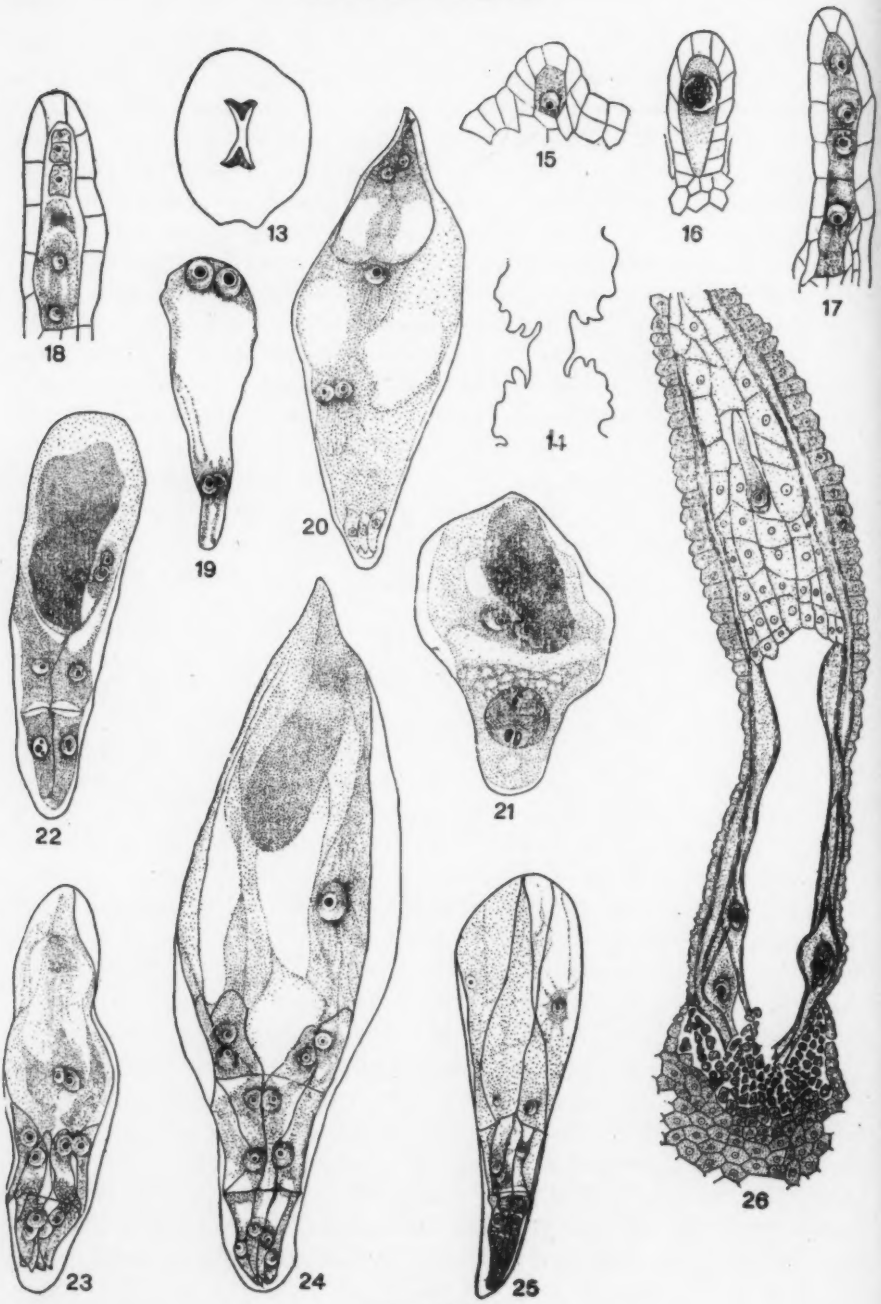
undergo further divisions in later stages. Most of the ovules turn through an angle of 180 degrees and become completely anatropous before the integuments are fully developed.

The nucellus in the initial stages of development of the ovule is composed of a few layers of cells. With the increase in size of the ovule, a multi-layered, massive and elongated nucellus results. It is interesting to note in this connection that generally from the four-nucleate stage of the female gametophyte onwards, disintegration of the nucellar epidermis starts. It proceeds from the micropylar end and extends towards the chalazal region, because of the maximum dilation of the gametophyte at the former end. At the mature embryo-sac stage, almost all of the nucellar cells become destroyed. The innermost cells of the integument which come in contact with the embryo-sac, elongate radially and form the endothecium. This has also been noted in other plants of the family by previous workers. It is further significant to note that at the chalazal end below the embryo-sac, a hypostase is formed. The vascular strand is unbranched and terminates at the chalazal region, a little above the hypostase.

Megasporogenesis

The single archesporial cell which differentiates in the hypodermal layer (Fig. 15) can be easily made out on account of its bigger size and prominent nucleus. It directly functions as the megaspore mother cell (Fig. 16). The position of the nucleus inside the megaspore mother cell is not fixed, as it has been found to lie towards the top or bottom or sometimes in the centre. It is interesting to note that Banerji (1940) noted a similar condition in *Costus speciosus*. On the completion of the usual reduction divisions, a linear tetrad of megaspores is formed (Fig. 17). The upper three megaspores degenerate and only the lower chalazal one becomes functional. The increase in size of the functional megaspore is accompanied with increased vacuolation. After the first meiotic division, the two daughter nuclei move apart to opposite poles and become embedded in a mass of copious cytoplasm (Fig. 18).

The next division gives rise to the four-nucleate stage, when two nuclei are arranged at each pole in a dense cytoplasmic mass. During this period, a large central vacuole occupies the central region of the sac. It may be mentioned that the nuclei lying towards the micropylar region are comparatively larger than those occupying the chalazal end. The embryo-sac at this stage becomes dilated at the micropylar end, and gradually tapers towards the chalaza. It becomes more or less club-like in appearance (Fig. 19). Division of the four nuclei gives rise to the eight-nucleate embryo-sac of the



FIGS. 13–26

TEXT-FIGS. 13–20. Fig. 13. Development of placenta, ×39. Fig. 14. Development and curvature of ovules, ×39. Fig. 15. Nucellus showing archesporial cell, ×475. Fig. 16. Megaspore mother cell, ×475. Fig. 17. Linear tetrad, ×475. Fig. 18. Two-nucleate embryo-sac with degenerating megaspores, ×475. Fig. 19. Four-nucleate embryo-sac, ×475. Fig. 20. Mature embryo-sac, ×475.

FIGS. 21–26. Fig. 21. Primary endosperm nucleus in division, ×475. Figs. 22–26. Stages in the development of the endosperm. Figs. 22–24, ×475. Figs. 25–26, ×300. Fig. 26. Chalazal haustorium of the endosperm; note zygote in resting condition.

Polygonum-type (Maheshwari, 1948). The mature embryo-sac is somewhat cylindrical in shape with maximum dilation near about the middle (Fig. 20). The two synergids are slightly hook-shaped in appearance with pointed apices and big basal vacuoles. Synergids of similar form have been recorded by Mauritzon (1935) in *Catalapa*, by Swamy (1941) in *Bignonia megapotamica* and by Govindu (1950) in four other genera. The egg-nucleus is much bigger in size than the synergid-nuclei. It is situated below the synergids. The polar nuclei fuse together to form the secondary nucleus of the embryo-sac. The antipodals are three in number and ephemeral in nature. The early degeneration of the antipodals has also been observed by Mauritzon (1935) and by Swamy (1941) but not by Govindu (1950) in *Jacaranda mimosæfolia*, *Parmentiera cerifera* and in *Tecoma stans*.

FERTILIZATION

Fertilization is porogamous. Plasmogamic condition of the egg and the male generative nucleus is delayed in comparison to that between the second male gamete and the fusion nucleus. The fusion of the egg and the sperm-nucleus does not appear to take place earlier than the 12-celled stage of the endosperm. The fertilized egg remains undivided for a long time. It divides after the division of the primary endosperm nucleus has taken place.

DEVELOPMENT OF THE ENDOSPERM

Before division, the primary endosperm nucleus migrates towards the chalazal region. It divides much earlier than the egg. Transverse wall-formation demarcates a small, basal, primary chalazal chamber and an upper large micropylar one (Fig. 21). The next division is synchronous and vertical in both the chambers, forming two tiers of two cells in each (Fig. 22). Subsequently, vertical walls are laid down in the two primary tiers at right angles to the previous plane of division. As a result, eight cells are formed with four cells in each tier (Fig. 23). The cells of the chalazal tier do not appear to divide further. They elongate enormously to form the highly conspicuous chalazal haustoria. The four cells of the micropylar tier usually divide transversely to form eight cells; thus the developing endosperm becomes composed of three tiers consisting of four cells each

(Fig. 24). By repeated divisions of the cells of the middle tier, an extensive endosperm tissue is formed (Fig. 25). The uppermost micropylar cells, formed towards the beginning of endosperm-development, gradually loose their protoplasmic contents and have no haustorial function (Fig. 25). The nucellar tissues in contact with the chalazal haustorium gradually disorganise. The cells below this degenerated zone in the chalazal part, arrange themselves in regular rows which are rich in protoplasmic contents and take deep stain (Fig. 26). Such radiating arrangement of cells at the chalazal region has been reported in *Bignonia megapotamica* by Swamy (1941).

The mature endosperm tissue, prior to division of the fertilised egg is composed of a large number of cells. These cells may be grouped as follows into four zones, based on the size, compactness and protoplasmic contents (Fig. 26).

(1) Four-celled haustorium at the chalazal region.

(2) Just above these, a compact mass of small, more or less rectangular, parenchymatous and prominently nucleated cells. These serve as proper endosperm in which the fertilised egg lie embedded before division.

(3) A mass of loosely arranged cells of larger size and lesser protoplasmic contents which stain feebly.

(4) Lastly, at the micropylar end, the remnants of broken walls of the disintegrated cells.

EMBRYOGENESIS

The fertilised egg lies embedded in the mature endosperm tissue. The zygote nucleus enlarges in size and migrates towards the lower part of the cell before division (Fig. 27). The first division of the zygote is transverse giving rise to a basal cell *Cb* and a terminal cell *Ca* (Fig. 28). The former divides transversely and the latter longitudinally, resulting in a ⊥-shaped pro-embryo composed of four cells (Fig. 29). Occasional occurrence of a linear row of four cells is also noticed (Fig. 30).

After the pro-embryo stage, the two upper cells divide transversely. These engender four quadrants disposed in a vertical plane (Fig. 31). The quadrants thus formed, next segment vertically to produce four upper octants and four lower ones (Fig. 32). The upper octants produce the cotyledonary portion of the embryo, undergoing first periclinal and then anticlinal divisions to separate the dermatogen (Fig. 33). Later, two layers of cells are differentiated beneath the epidermis. The lower octants produce the hypocotyledonary portion (Fig. 32).

The hypophysis originates from the cell *d*, a daughter cell of *m* (Figs. 33–35). The tissues produced by it are deeply embedded in the body of the embryo. To begin with, the hypophysis cell *h*, although similar in shape to the other cells of the suspensor, soon becomes somewhat rounded at the lower end. It divides transversely to form two daughter cells, each of which undergoes two divisions by walls at right angles to one another (Figs. 34–35). Of the resulting eight cells, the lowest four form the initials of the root cortex and the upper four produce the root cap and the root epidermis.

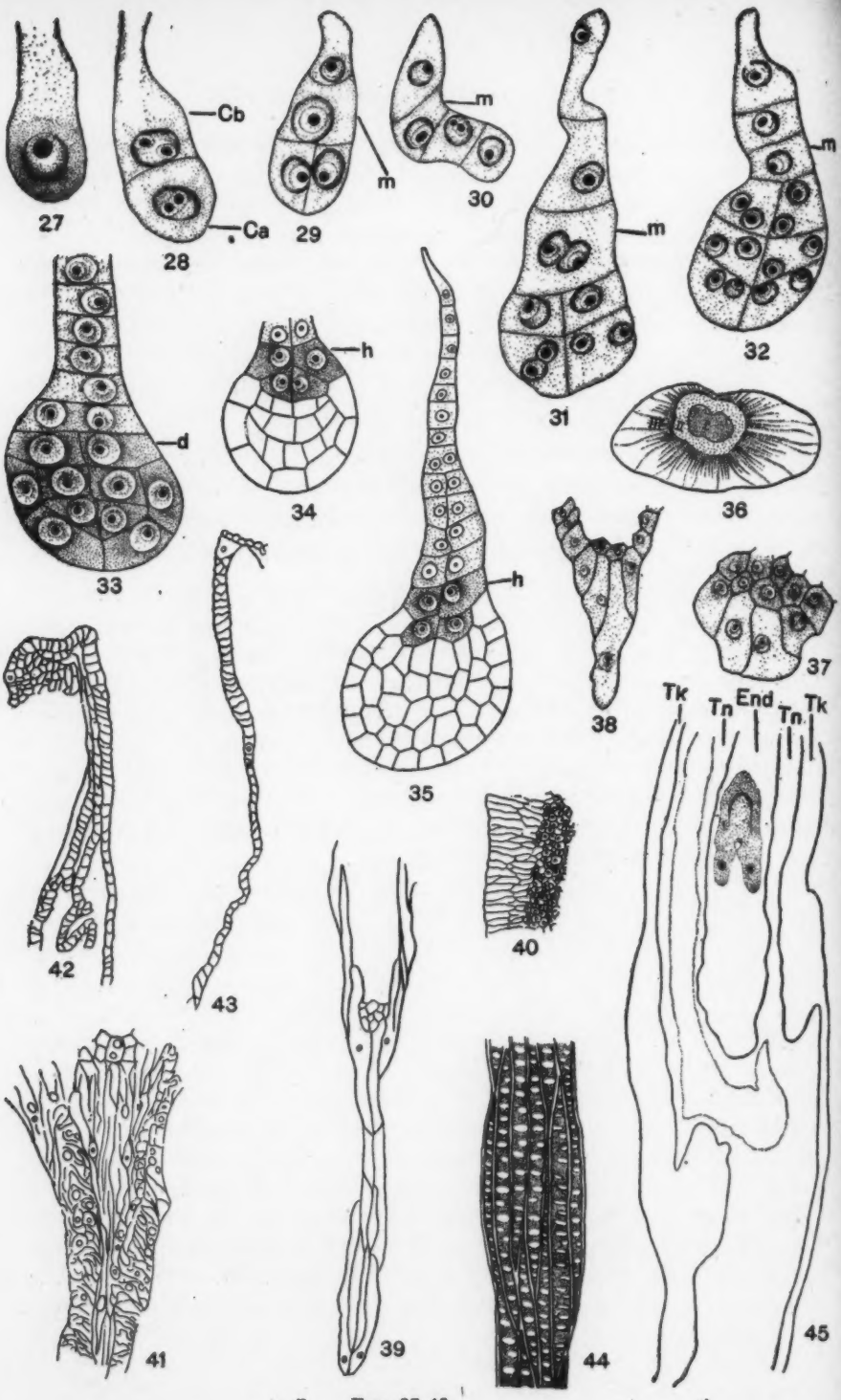
The suspensor is long and filamentous. At maturity it consists of about fifteen cells, the lowest few of which divide lengthwise. Each cell thus produced has a prominent nucleus and is longer than broad (Fig. 35).

In the mature seed, the cotyledons are large, massive, more or less obcordate in shape (Fig. 36) and completely fill up the seed.

Development of the Wing

The seeds of *Oroxylum indicum* are characterised by the presence of a thin, membranous structure called the "Wing". It forms an outer fringe, composed of a single layer of mostly thin-walled cells. Its maximum extension is on the two lateral sides of the seed. The delicate, almost transparent, fringe is supported on brownish, rib-like radiating strands composed of thick-walled cells (Zones III and I in Fig. 36), which encircle the cotyledons completely .In between these two zones, the area of the wing is occupied mainly with thick-walled cells and partly with thin-walled ones (Zone II in Fig. 36). That the thickening appears to be due to deposition of cellulose has been ascertained by microchemical tests.

The early stages in the development of the wing is indicated by the elongation and increase in the size of the two tip-cells at the chalazal end of the ovule before fertilization (Fig. 37). The process becomes accentuated after syngamy (Fig. 38). During the initial stages of the development of the endosperm, the chalazal tip-cells and the lateral epidermal cells of the integument divide both longitudinally and transversely and become more active. This is accompanied by an elongation of these cells. As a result broad, multicellular outgrowths are formed surrounding the ovules during the earlier stages of endosperm development. The cells produced from the chalazal tip elongate rapidly as slender columns, closely adpressed with each other. After attaining considerable length, these become curved and interwined (Fig. 39). The development of the wing thus proceeds mainly on two aspects: (1) from the chalazal tip and (2) from the outermost integumentary cells on the lateral sides of the ovules (Fig. 40). These two zones composed of thin-walled cells ultimately coalesce forming the major part


27
Cb
Ca
28
29
m
30
m
31
m
32
h
34
d
33
h
35
36
37
38
Tk
Tn
End
Tn
Tk
42
43
40
41
39
44
45

FIGS. 27–45

TEXT-FIGS. 27–35. Stages in the development of the embryo. Figs. 27–33, ×550. Figs. 34–35, ×300.

TEXT-FIGS. 36–45. Fig. 36. Mature winged seed, ×½. Figs. 37–39. Chalazal tip-cells of the ovule elongating. Figs. 37–38, ×300. Fig. 39, ×77. Fig. 40. Lateral integumentary cells of the ovule dividing, ×77. Fig. 41. Intermingling of integumentary strands from the chalazal end and the lateral sides, ×300. Fig. 42. Micropylar strands, ×75. Fig. 43. Funicular strand, ×75. Fig. 44. Pitted thickenings in the inner cells of the wing, ×475. Fig. 45. Distribution of thick-walled tissue, remnant of endosperm and mature embryo, ×23. *Tk.*, Thick-walled; *Tn.*, Thin-walled; *End.*, Endosperm.

of the fringe of the mature wing (Fig. 41). The integumentary cells of the micropylar end are less active during this period. These begin to increase in number first laterally and then terminally towards the later stages of endosperm development (Fig. 42). Multicellular, strand-like outgrowths are also produced from the funicular base (Fig. 43). These micropylar and funicular outgrowths overlap the filamentous strands already produced as described earlier. All these cells appear to be thin-walled and constitute the fringe of the wing.

Thickenings of various types, mostly with pits, appear in the long, fibre-like inner (Fig. 44) integumentary cells during the later stages of the development of the embryo. Thickenings start from the chalazal and the micropylar ends simultaneously. The deposition of additional cellulose then proceeds upwards and downwards from the chalazal and the micropylar ends respectively. Finally the multi-layered thickened cells completely encircle the mature embryo (Fig. 45). This thick-walled tissue lies innermost to the thin-walled cells previously formed and radiates in the midst of the latter. As a result, in the mature seed, the central part of the wing surrounding the cotyledons (Zone I in the Fig. 36) is only composed of thick-walled cells. Occasional radiations emanate from this zone rendering a ribbed appearance to the fully-formed wing.

CONCLUSION

The somatic chromosome number of *Oroxylum indicum* as determined in the course of the present investigation is not in conformity with observation of Venkatasubban (1945). He mentions $2n = 30$, but does not give gametic confirmation. No information regarding the morphology of the somatic chromosomes of *Oroxylum indicum* is available in his report. So far as the present writer is aware, apart from Venkatasubban, no other investigator has recorded the chromosome-morphology of *Oroxylum indicum*. According to him the genera approaching *Oroxylum* in chromosome numbers are *Spathodea* with $2n = 26$ and *Millingtonia* with $2n = 30$.

Venkatasubban (1945) observed multinucleate condition and intrusive nature of the tapetal cells in *Dolichondrone rheedii* which agree in the observations made in the present material.

Erdtman (1952) gives a full account of the morphology of the pollen grains of Bignoniaceæ. It is interesting to note that the broad characteristics of the pollen grains of *Oroxylum indicum* agree in general with those observed by others in the family Bignoniaceæ.

Based on the floral evolution among the Sympetalæ (Wernham, 1913), the origin of the Bignoniaceæ has been traced from the apocynal plexus and it is placed among the Tubifloræ. The dendroid habit and woody follicles in some members of the Bignoniaceæ are put forth as reminescent of similar conditions which predominate in the Apocynaceæ. When the embryological findings of both the families are considered, the differences are so wide apart that the two families seem to be far removed.

On the contrary, Bignoniaceæ resemble more clearly the families like Verbenaceæ and Boraginaceæ which are all included under Engler's Tubifloræ and under the Personales by Bentham and Hooker (1862–67) and also by Hutchinson (1926).

Each of these families has cellular endosperm, whereas the endosperm of the Apocynaceæ is nuclear and becomes cellular only at later stages. As observed in the course of this investigation and also recorded by all other previous workers, in the family Bignoniaceæ, one of the most noteworthy features in the embryology is the development of the endosperm tissue.

In *Oroxylum indicum*, the early development of the endosperm upto the 4-celled stage follows the *Catalapa* type of Mauritzon (1935). But it differs in the more active, important and aggressive role of the chalazal haustorium. The haustorial activity of the chalazal cells of *Tecoma stans* and *Jacaranda mimosæfolia* (Govindu, 1950) resembles that of *Oroxylum indicum*. But *Tecoma stans* (Govindu, 1950) differs from the other two genera in having only two cells at the micropylar end instead of four. *Oroxylum indicum* differs from *Bignonia megapotamica* (Swamy, 1941) and *Parmentiera cerifera* (Govindu, 1950) because the latter two genera have only two cells composing the chalazal haustorium.

In *Oroxylum indicum* the inconspicuousness of haustorial activity of the topmost micropylar cells is a characteristic feature. The pronounced haustorial activity of the micropylar cells of *Catalapa bignonioides* (Mauritzon, 1935) may be stated to have become reversed in *Jacaranda mimosæfolia* (Govindu, 1950) and in *Oroxylum indicum*. It is further

interesting to note that the last trace of insignificant haustorial activity of the four cells at the micropylar region of *Jacaranda mimosæfolia* (Govindu, 1950) has been lost altogether in *Oroxylum indicum*.

The observations on the development of embryo of *Oroxylum indicum* confirm those of Soueges (1940) and probably of Swamy (1941). The variations in the total number of suspensor cells as well as in the vertical division of the lower ones of *Oroxylum indicum* are not recorded by the previous workers.

The development and the structure of the wing on the seeds of *Oroxylum indicum* follow in general outline those of *Jacaranda mimosæfolia* and *Tecoma stans* (Govindu, 1950) which, however, have not been studied in detail. Moreover, the thickenings on the inner cells of the wing of *Oroxylum indicum* appear to be due to heavy deposition of cellulose and not of lignin as noted in *Tecoma stans* by Govindu (1950).

Summary

The paper gives an account of the cytological and embryological investigations on *Oroxylum indicum*.

(1) The diploid number of chromosomes is 28. A detailed morphological study of the somatic chromosomes shows the presence of one pair of long chromosomes, eight pairs of medium-sized chromosomes and five pairs of short chromosomes. There are five pairs of chromosomes with secondary constrictions of which three medium-sized pairs are with satellites.

(2) Meiosis is normal and 14 bivalent chromosomes have been recorded. Pollen-formation is of the simultaneous type. The pollen tetrads are mostly tetrahedral in arrangement; sometimes decussate tetrads have been noted. The pollen grains are binucleate, spheroidal-prolate, tricolpate with sexine thicker than the nexine.

(3) The anther-wall at maturity is made up of the cutinised epidermis, generally three-layered endothecium with fibrous bands and a minimum of four middle layers.

(4) The tapetum is of the secretory type. The tapetal cells are 2 to 4 nucleate.

(5) The ovules are unitegmic, tenuinucellate and mostly anatropous. Hemitropous ovules have also been observed.

(6) The single archesporial cell is hypodermal in origin and functions directly as the megaspore mother cell which undergoes the reduction divisions and produces a monosporic embryo-sac of the "Polygonum type".

(7) Fertilization is porogamous. Stages of fertilization have been observed.

(8) The endosperm is cellular from the beginning and corresponds to the *Catalapa* type of Mauritzon (1935) with minor variations. The four-celled chalazal haustorium is functional.

(9) The development of the embryo is primarily of the *Capsella* type with secondary variations commonly observed in members of Solanaceæ.

(10) The outer integumentary cells of the ovule take part in the development of the wings on the seeds. At first the chalazal tip-cells elongate, divide both longitudinally and transversely and form columnar strands. These intermingle with tissues formed later by the cells on the lateral sides, of the micropylar end and also on the funicular part of the fertilised ovule.

At a later stage cellulose thickenings, mostly with pits, are laid down on the inner cells of the wings.

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LITERATURE CITED

Banerjee, I. .. "A contribution to the life-history of *Costus speciosus* Smith," *J. Indian. bot. Soc.*, 1940, **19**, 181–96.

———— .. "Morphological and Cytological studies on *Citrus grandis* Osbeck," *Phytomorphology*, 1954, **4**, 390–96.

Bentham, G. and Hooker, J. D. *Genera Plantarum, London*, 1862–67.

Conagin, Candida, H. T. .. "Paradichlorobenzene for chromosome count," *Stain Tech.*, 1951, **26**, 276.

Darlington, C. D. and Janaki Ammal, E. K. .. *Chromosome Atlas of Cultivated Plants*, 1945.

Engler, A. and Prantl, K. .. *Die natürlichen pflanzen-familien, Jena*, 1897.

Erdtman, G. .. *Pollen Morphology and Plant Taxonomy, Angiosperms*, 1952.

Govindu, H. C. .. "Studies in the embryology of some members of the Bignoniaceæ," *Proc. Ind. Acad. Sci.*, 1950, **32**, 164–78.

Hooker, J. D. .. *Flora of British India*, 1894. **4**.

Hutchinson, J. .. *Families of Flowering Plants, I.* London, 1926.

Johansen .. *Plant Embryology*, 1950.

Maheshwari, P. .. "A critical review of the types of embryo-sacs in Angiosperms," *New Phyt.*, 1937, **36**, 359–417.

———— .. "Recent work of the types of embryo-sacs in Angiosperms," *J. Indian bot. Soc.*, 1941, **20**, 229–61.

———— .. *An Introduction to the Embryology of Angiosperms*, 1950.

Mauritzon, J. .. "Etwas über die Embryologie der Bignoniaceen," *Bot. Not.*, 1935.

Meyer, James, R. .. "Prefixing with paradichlorobenzene to facilitate chromosome study," *Stain Tech.*, 1945, **20**, 121–25.

Prain, D. .. *Bengal Plants*, 1903, **2.**

Raghavan, T. S. and Venkatasubban, K. R. "Studies in the Bignoniaceæ, *I.*" *J. Indian bot. Soc.*, 1940, **19**, 293–98.

Rendle, A. D. .. *Classification of Flowering Plants*, 1930, **2.**

Schnarf, K. .. *Vergleichende Embryologie der Angiospermen, Berlin*, 1931.

Sharma, A. K. and Miss Chitra Ghosh "Oxyquinoline—its possibilities in other cytological procedures," *Sci. and Cult.*, 1951, **16**, 528–30.

—— and Mookherjee, A. .. "Paradichlorobenzene and other chemicals in chromosome work," *Stain Tech.*, 1955, **30**, 1–7.

Soueges, R. .. "Embryogènie des Bignoniaceen. Development de lèmbryon chez le *Catalapa kæmpferi* Sieb. et. Zuce," *Comp. Rend. Acad. Sci.* (*Paris*), 1940, **210**, 116–18.

Swamy, B. G. L. .. "Contributions to the life-history of *Bignonia megapotamica*," *J. Indian bot. Soc.*, 1941, **20**, 299–305.

Tjio, J. H. and Levan, A. .. "The use of oxyquinoline in chromosome analysis," *Anal. Estac. Exp. Aula Die.*, 1950, **2**, 21.

Venkatasubban, K. R. .. "Cytological studies in Bignoniaceæ," *Proc. Ind. Acad. Sci.*, 1945, **21 B**, 77–92.

Wang, D. T. .. "Karyokinetic study on *Catalapa bungei*," *Bull. Fan. Mem. Inst. Biol.* (*Bot.*) *Peiping*, 1940, **10** (3), 185–88.

Wernham, H. F. .. "Floral evolution with particular reference to the Sympetalous dicotyledons", *New Phytl.*, 1913.

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MUSCULAR CONTRACTION AND RELAXATION*

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AND

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When a muscle contracts, it undergoes several changes such as mechanical, thermal, chemical, volume changes, changes in hydrogen-ion concentration, electric, osmotic, changes in X-ray diffraction, etc. Various attempts have been made to correlate these phenomena in order to elucidate the intimate nature of muscular contraction.

Two fundamentally opposed theories are advanced to explain the contraction of muscle. In the first, contraction is considered to be due to release of mechanical energy previously stored in the structure, relaxation being therefore, considered to be an active phenomenon. In the second, the chemical energy is supposed to be released by the stimulation to enable the muscle to do work, relaxation being considered as a passive phenomenon.

The proponents of these views have been quite emphatic in their assertions. Hill (1950) writes about active relaxation, that "such theories have really out-lived their usefulness," and Ramsey (1955) entirely disagrees with this view. Hill criticizes the active relaxation hypothesis on the basis of his demonstration that heat production precedes mechanical activity when a muscle is stimulated and the heat of relaxation is nil. According to Ramsey, this does not provide evidence against the hypothesis, because all of the reactions cannot be specified. He states that the fact that heat precedes mechanical activity suggests that it is unrelated to contraction, since any free energy liberated by the process of mechanical activity would have to be used simultaneously with the energy fraction degraded as heat, so that the fact that heat precedes mechanical activity, does not contradict the hypothesis of active relaxation. That the heat of relaxation is nil may mean that the cycle during relaxation is isothermal.

Since the facts and arguments in support of these views are well known, we shall therefore limit the present discussion to presentation of our own experiments and views. We have come to the conclusion that in unstriated

* A discussion on "Muscular contraction and relaxation" at the meeting of the Physiological Society of India, held at Agra.

muscle, there are both active and passive relaxations (Singh and Singh, 1950 *a*).

The above view is based on the following observations. A criterion of active relaxation accepted by both sides, is that relaxation of unloaded muscle must be shown to occur. This has been found to occur in certain muscles. Thus unloaded guinea pig's uterus and frog's stomach muscle relax when treated with adrenaline. Dog's stomach muscle relaxes after stimulation with alternating current, potassium or acetylcholine. A convincing example of relaxation of unloaded muscle is that shown by the retractor of the introvert of the marine worm Phascolosoma. This muscle differs from other muscles mentioned above, that the unloaded muscle relaxes comparatively with great speed and to a greater extent. But sometimes, these muscles do not relax when unloaded, but readily do so when loaded. Rabbit's gut muscle is very sensitive to adrenaline and relaxes when loaded but not when unloaded. *Mytilus* muscle also does not relax when unloaded.

Though relaxation of unloaded muscle has been shown to occur, the question arises, as to why it occurs in some muscles and why only sometimes in other muscles. This therefore leads to the conclusion, that there are both active and passive relaxations. This conclusion is supported by the differential action of substances, which affect the two kinds of relaxation differently. The differential action of substances is studied to elucidate the nature of relaxation of unloaded muscle. It may be due to pulling on the muscle by internal elastic structures; the muscle would then be loaded and relaxation passive, though apparently the muscle is unloaded. To know whether the relaxation of unloaded muscle is active or passive, we have to compare the behaviour of loaded and unloaded muscle towards certain agencies.

These agencies interfere with metabolism in some way or other, and have a differential effect on the two kinds of relaxation. If the muscle (guinea pig's uterus, hen's duodenum, frog's stomach muscle) is asphyxiated or treated with sodium cyanide (1 in 10,000), then loaded muscle may relax profoundly, but unloaded muscle does not relax; on the contrary it may contract. Similar effects may be produced with sodium azide or iodoacetic acid. These experiments would therefore dispose off the objection that relaxation of unloaded muscle might be caused by stress in the internal elastic structures, as otherwise the unloaded muscle would also be relaxed by these agents.

This view is supported by further experiments in which the relaxation of unloaded frog's stomach muscle is produced by adrenaline. These

experiments are conducted in four steps: (1) Relaxation of unloaded muscle is produced by adrenaline; the muscle is then returned to adrenaline-free saline. (2) The muscle is treated with cyanide and adrenaline is found to have lost its effect; the muscle is then returned to adrenaline-free saline, which contains cyanide. (3) The muscle is now treated with saline containing both cyanide and glucose; adrenaline again causes relaxation. (4) The muscle is returned to adrenaline-free saline containing cyanide, glucose and iodoacetic acid; adrenaline no more produces any relaxation. These experiments leave no doubt as to the active nature of the relaxation process in some unstriated muscles (Singh and Singh, 1952). Also, there is no doubt that passive relaxation occurs, if active relaxation is inhibited. In unstriated muscle, therefore, there are two systems; in one the relaxation is active and in the other passive.

In striated muscle, there have been some controversial observations. The difference in the technique of the two sets of observers is that in one case (Ramsey and Street, 1941), the muscle was unloaded, and in the other case (Hill, 1949), the muscle was loaded. It is possible, that when the muscle is loaded, then active relaxation is absent. Some experiments suggest such an inference (Singh and Singh, 1950 *b*, 1955). Thus some substance cause relaxation of unloaded muscle, but not when it is loaded. Thus there is antagonism between active and passive relaxation.

If unstriated muscle is stretched, then some change is produced that increases the tendency to contraction and diminishes that to relaxation, hence the antagonism between active and passive relaxation. This reaction to stretch diminishes the inhibitory action of substances and converts it into an excitatory one. Stretching or increase in initial length is thus one of the factors in drug reversal.

It would appear that both the excitatory and the contractile systems are affected by stretch. The involvement of the excitatory system is shown by the fact that stretching may increase the tone which is susceptible to asphyxia. This tone therefore has an optimum length for its maintainance. The involvement of the contractile system is shown by the fact that the effects of stretching occur in dying or heat-killed muscle. The response of the heat-killed muscle is augmented by increase in initial length. It would appear that both systems in unstriated muscle become more active if the initial length of the muscle is increased.

The increased response of the excitatory system to stretch would entail increased expenditure of energy with increased load. The involvement of

the contractile system would curtail this increased expenditure of energy with load, and would thus enable the muscle to respond with greater economy.

It is often said, that contraction of muscle may be complicated by simultaneous relaxation, and attempts have been made to study the contraction of muscle unaccompanied by relaxation. That such a possibility occurs is shown by the behaviour of heat-killed muscle. If this muscle is heated, then a certain tension is produced, but if active relaxation is inactivated by heating to 70° C., then the tension produced is greater than previously, if the muscle is heated to the same temperature as before. The tension produced in the former case was therefore less, because the muscle was contracting and relaxing at the same time.

This is also shown by the paradoxical reaction, in which oxygen or glucose inhibit the mechanical response, which means that increased expenditure of energy results in diminution of the response. This shows that during contraction, an active process was taking place which diminished the mechanical response, and this could only be active relaxation. The augmentation of the mechanical response by asphyxia, iodoacetic acid or cyanide would therefore be due to inhibition of this active process. The mechanical response is at first augmented by asphyxia and then declines. This suggests that active relaxation is more susceptible to asphyxia than active contraction. This is actually found to be true experimentally.

The simultaneous contraction and relaxation of muscle implies that the mechano-chemical coupling for relaxation would occur at the same time as that for contraction; it is like purchasing a return ticket. Contraction and relaxation would be energised as suggested by us in unstriated muscle, but no thermal changes would be detectable during relaxation. The absence of heat production during relaxation, therefore, does not imply the absence of active relaxation. Even if contraction is not energised some chemical changes, and therefore heat production, might occur prior to any mechanical change to start off the machine, even if the relaxation was active. Further relaxation may be energised during contraction, but may remain latent till the contraction is over or nearly over. Under these conditions, no heat production would be detected during relaxation.

In striated muscle, Ramsey and Street have found relaxation of unloaded single muscle fibres. We stimulated unloaded frog's rectus abdominis with potassium and acetylcholine, but did not observe any active relaxation, in marked contrast to dog's stomach muscle which relaxes actively, when so stimulated. This may be due to some sort of inhibition of active relaxation in the whole muscle. In this connexion, we may mention some of our other

results. We have not found relaxation of unloaded *Mytilus* muscle; no heat production has been found in this muscle during relaxation (Abbott and Lowy, 1955). Increased lactic acid production may occur during relaxation of unstriated muscle (Bharadwaj and Singh, 1951; Mohme-Lundholm, 1953).

Next, let us take some aspects of contraction of muscle. According to modern views, denaturation consists of an alteration of the specific internal structure of the protein wherein the closely folded peptide chains unfold. Similarly, the contraction of muscle is supposed to be due to folding of the contractile protein; so relaxation would be due to unfolding of the muscle proteins. Thus according to these views, the process of relaxation would be similar to denaturation of proteins. Experimentally it has been found that many agencies which denature proteins, also cause active relaxation of the contractile mechanism. These experiments throw light both on the process of denaturation and the mechanism of muscular contraction.

During denaturation it is assumed, that there is a rearrangement of the peptide chains in the protein molecule due to rupture by denaturing agents of the weak bonds. During relaxation of a tonic contraction, there must be rupture of such bonds, so that tonic contraction in unstriated muscle is maintained by cross linkages between the folded polypeptide chains. This is supported by the fact that coagulated muscle contracts and exerts considerable tension. Coagulation is supposed to be due to the formation of intermolecular salt bridges between ionic groups which are displaced to the surface of the particle through the unfolding of the polypeptide chains.

By the action of reagents which cause active or passive relaxation of heat-killed muscle, it is concluded that tonus in unstriated muscle is maintained by disulphide linkages, hydrogen bonds and salt linkages. Dissolution of the former two, results in active relaxation, and dissolution of the last, in passive relaxation. By the action of these reagents, the nature of the contractile mechanism is ascertained. The contractile mechanism of *Mytilus* muscle is thus found to be different from frog or mammalian unstriated muscle. The rupture of these tonic linkages during phasic contraction, presumably cause latency relaxation.

The unfolding of the polypeptide chains during relaxation is suggested by another experiment. The effects of stretching and relaxation are similar (Singh and Singh, 1955). As stretching is known to unfold fibrous proteins, this suggests that same happens during relaxation.

In striated muscle also there appear to be two systems, in one the relaxation being active, and in the other passive. This is suggested by the

fact that active relaxation occurs in unloaded single muscle fibres, but not in the whole muscle. In the latter, therefore, active relaxation appears to be inhibited and passive relaxation occurs, when active relaxation does not occur.

The existence of two systems in some unstriated muscles can be shown directly. Thus if unloaded heat-killed muscle is heated to 60–70° C., then it relaxes. Hence the supply of energy to the contractile mechanism causes active relaxation. If once the muscle is heated to 70° C., then active relaxation is inactivated. It now gives a contraction which is proportional to rise in temperature, and the relaxation is passive. Thus supply of energy to the contractile mechanism causes a proportionate contraction. In some unstriated muscles therefore, both contraction and relaxation are energised.

REFERENCES

Abbott, B. C. and Lowy, J. .. *J. Physiol.*, 1955, **130**, 25.

Bharadwaj, U. R. and Singh, I. *Proc. Ind. Acad. Sci.*, 1951, **33**, 257.

Hill, A. V. .. *Proc. Roy. Soc.*, 1949, **136 B**, 429.

Mohme-Lundholm .. *Acta Physiol. Scandinav. Suppl.*, 1953, **29**, 108. Quoted from R. F. Furchgott, *Pharmacol. Rev.*, 1955, **7**, 183.

Ramsey, R. W. and Street, S. F. *Biol. Symp.*, 1941, **3**, 9.

Ramsey, R. W. .. *Amer. J. Physiol.*, 1955, **181**, 688.

Singh, S. I. and Singh, I. .. *Nature*, 1950 *a*, **166**, 647.

———— .. *Curr. Sci.*, 1952, **21**, 77.

———— .. *Proc. Ind. Acad. Sci.*, 1950 *b*, **31**, 351.

———— .. *Ibid.*, 1955.

THE LIFE-HISTORY OF *PUCCINIA ROMAGNOLIANA* MAIRE AND SACC.*

By T. S. Ramakrishnan, F.A.Sc. and N. V. Sundaram

Received January 5, 1956

Puccinia romagnoliana is widely distributed in many parts of the world. In India it has been recorded from almost all the states. It is prevalent on many species of *Cyperus*. *C. rotundus* is the most common host. It has also been observed on *C. tegetum* Roxb., *C. arenarius* Retz., *C. tuberosus* Rottb., *C. capitatus* (= *C. aristatus* Rottb.), *C. compressus* L. and some unidentified species of *Cyperus*. Though the rust enjoyed a wide distribution and host range the uredial and the telial stages alone were known till now. In recent years while a survey was being conducted on the incidence of rusts in Coimbatore by the junior author it was noticed that by the side of heavily infected plants of *C. rotundus*, æcia were developing on the leaves of *Ipomæa rumicifolia* Choisy and *I. pestigridis* L. This suggested that there may be some connection between the rusts on the different hosts. In order to clarify the relationship, inoculation experiments were conducted and the heteroecious nature of *P. romagnoliana* was established, the two species of *Ipomæa* playing the role of alternate hosts. The results of these studies are embodied in this communication.

Uredia

The uredia are oval or round, yellow in colour and formed hypophyllously in the centre of brown rust spots. The leaf-sheath and the culm are also liable to be infected. In Coimbatore the sori are formed throughout the year but are very conspicuous during the months of August to February. Humid conditions favour the prevalence of the rust. The uredia are subepidermal and burst through the epidermis exposing the powdery mass of spores.

The urediospores are pedicellate, borne on hyaline pedicels, oval to subglobose, measuring $22 \times 19\,\mu$ (19–28 × 12–22), wall coloured brown, aculeate and with two equatorial germ pores. They germinate readily in water in six to eight hours, producing long and unbranched germ tubes. The viability of the spores is however lost in four months, when stored under laboratory conditions.

* This formed part of the thesis for the M.Sc. Degree of the Madras University by the Junior author under the guidance of the senior author.

The leaves of *Cyperus rotundus* and *C. bulbosus* Vahl. were inoculated with viable urediospores. Successful infection was obtained on the former alone. Uredia developed in 19 to 23 days after inoculation. Infection took place through the stomata on the lower surface of the leaves. There was no evidence of infection when the inoculation was carried out on the upper surface alone.

TELIA

Telia are found mixed with the uredia. They are hypophyllous, occurring singly or in groups, forming dark patches. They are subepidermal and remain covered by the epidermis for a long period, though eventually the covering is ruptured and the spores are exposed. The telia are elongated, measuring one to five millimeters in length and half to two millimeters in breadth. Each telium has one or more compartments separated by closely packed clusters of vertically elongated cells (which must be considered to be paraphyses). The teliospores have coloured stalks which vary in length, extending up to 68 μ. The spores are oblong or club-shaped, smoothwalled, two-celled, constricted at the septum and measure $47 \times 19\,\mu$ ($28–68 \times 12–25$). The apex is round, flattened or obtuse and thickened up to 12 μ. The two cells of the spore are either equal in length or the lower one is longer and tapering towards the base. Mesospores are common. These measure $28–53 \times 12–19\,\mu$ and are either obtuse or flattened at the apex which may be thickened up to 12 μ.

The teliospores germinate when mature without a rest period. Germination was obtained for four months after collection. Later trials were not successful. Even fresh spores exhibited only about 50 per cent. germination. One or two basidia are formed from each spore. The basidium is three-septate and one ovate or subglobose hyaline basidiospore is produced from each cell, borne on a sterigma. Sometimes elongated fine hyphal growths are formed instead of the basidiospores.

PYCNIA AND AECIA

In nature these have been observed on the leaves of *I. rumicifolia* and *I. pestigridis*. The affected parts are hypertrophied forming slightly thickened concavo-convex yellowish infection spots, the convex side being towards the lower surface. Pycnia are mainly epiphyllous, minute and orange coloured. They are sub-epidermal, flask-shaped, 140–230 μ high and 120–170 μ broad, with a bundle of paraphyses protruding through the ostiole; the pycniospores are elliptic, subhyaline or light orange coloured. The æcia are formed hypophyllously in groups, bright orange coloured and cupulate with recurved lacerated peridia. They measure 280–440 μ in width and

260–330 μ in height. The peridial cells are hyaline, polygonal, thick-walled with the inner wall thickly and strongly verrucose and measure 25–34 × 19–25 μ. The æciospores are catenulate, subglobose, thin-walled, wall hyaline and finely verruculose, contents granular and orange coloured and measure 19 × 16 μ (16–24 × 12–17). Fresh æciospores germinate readily producing long germ tubes.

Infection Experiments

In order to find out whether any relationship existed between the rusts on *Cyperus* and *Ipomæa* several cross-inoculations were carried out. Healthy plants of *I. rumicifolia* were raised in pots in the green house. The leaves of these plants were inoculated with germinating teliospores. After inoculation the plants were covered with alkathene bags to maintain high humidity. On the sixth day round yellow infection spots were evident in the inoculated portions. Pycnia could be distinguished as minute orange coloured dots on the upper surface on the eigth day. In the meantime the infected portions became hypertrophied developing a bulge on the lower surface. On the eleventh day young orange coloured æcia protruded on the lower surface. Two days later these opened out exposing the bright orange mass of spores.

To complete the life-cycle, fresh æciospores were inoculated on healthy plants of *C. rotundus* which were then covered with alkathene bags. On the twenty-second day typical uredia developed on the inoculated leaves. Thus the life-cycle was completed and it was established that *I. rumicifolia* functioned as the alternate host of *P. romagnoliana.*

Unconnected æcia have been reported on other species of *Ipomæa. A. kærnbachii* P. Henn. has been recorded on *I. reptans* (= *I. aquatica*), *I. pescapræ* (= *I. biloba*) and *I. hederacea* (Butler and Bisby, 1931). To find out whether any connection existed between these, inoculations were made with germinating teliospores of *P. romagnoliana* on the different species of *Ipomæa* available at Coimbatore. The plants were raised in an isolated place and were covered with alkathene bags soon after inoculation. Suitable controls were maintained in all cases. The following results were obtained.

The first two plants included in the above trials have been recorded as hosts for *A. kærnbachii* P. Henn. But these were not infected by the germinating teliospores of *P. romagnoliana.* The dimensions of the æciospores of the former were found to be identical with those of the latter. It appears that æcidia of more than one rust are developed on *Ipomæa* though they resemble each other. The æciospores from *I. pestigridis* and *I. rumicifolia* readily infected the leaves of *Cyperus rotundus.* This was confirmed by repeated inoculations.

TABLE I

Results of inoculation

Host	Part inoculated	No. inoculated	No. infected	Incubation period in days	
				Pycnial formation	Aecial formation
Ipomæa reptans ..	Leaves	20	Nil	..	..
Ipomæa pescapræ ..	,,	20	,,	..	..
Ipomæa pestigridis ..	,,	20	20	9	12
	Bracts	15	15	9	12
Ipomæa rumicifolia ..	Leaves	20	20	8	11–12

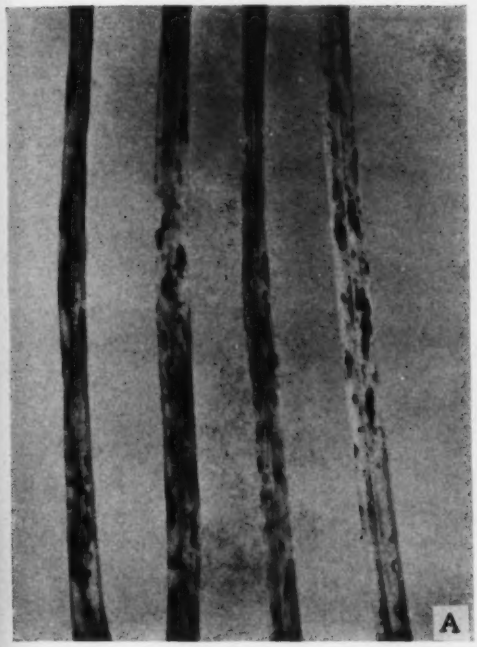
EMENDED DESCRIPTION OF THE RUST

Since the alternate host of the rust has been discovered it has become necessary to provide an amended description of the rust including all the stages of its development and this is given hereunder.

Pycnia and æcia on *Ipomæa rumicifolia* and *I. pestigridis*; uredia and telia on *Cyperus rotundus* and some other species:

Pycnia and æcia.—Rust spots hypertrophied, amphigenous, round, yellowish; pycnia mostly epiphyllous, orange coloured, subepidermal, flask-shaped, with protruding paraphyses, 140–230 × 120–170 μ, pycniospores elliptic and subhyaline or light orange coloured; æcia hypophyllous, cupulate, with recurved lacerated peridium, 280–440 × 260–330 μ; peridial cells hyaline, polygonal, inner wall densely and strongly verrucose, 25–34 × 19–25 μ; æciospores catenulate, subglobose, thin-walled, wall hyaline and finely verruculose, contents orange coloured, 19 × 16 μ (16–24 × 12–17).

Uredia and telia.—Uredia on leaf-blades leaf-sheath and culms, hypophyllous on the leaf-blades, elongated or oval, 1–3 mm. × 0·5–1 mm., subepidermal, erumpent, pulverulent, brown; urediospores subglobose or obovate, aculeate, with two equatorial germ pores, 22 × 19 μ (19–28 × 12–22); telia hypophyllous, mixed with the uredia, subepidermal, long covered by the epidermis, loculate with one or more locules bounded by closely packed vertically arranged paraphyses; teliospores two-celled, oblong to clavate, smooth-walled, constricted at the septum, apex rounded or obtuse and thickened up to 12 μ, brown measuring 47 × 19 μ (28–68 × 12–25), pedicellate,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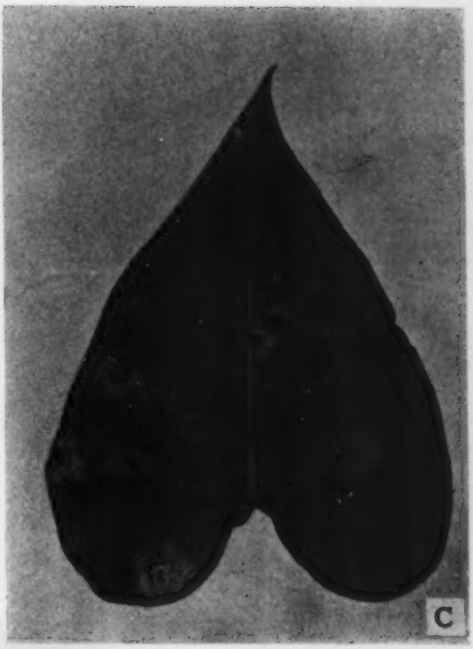
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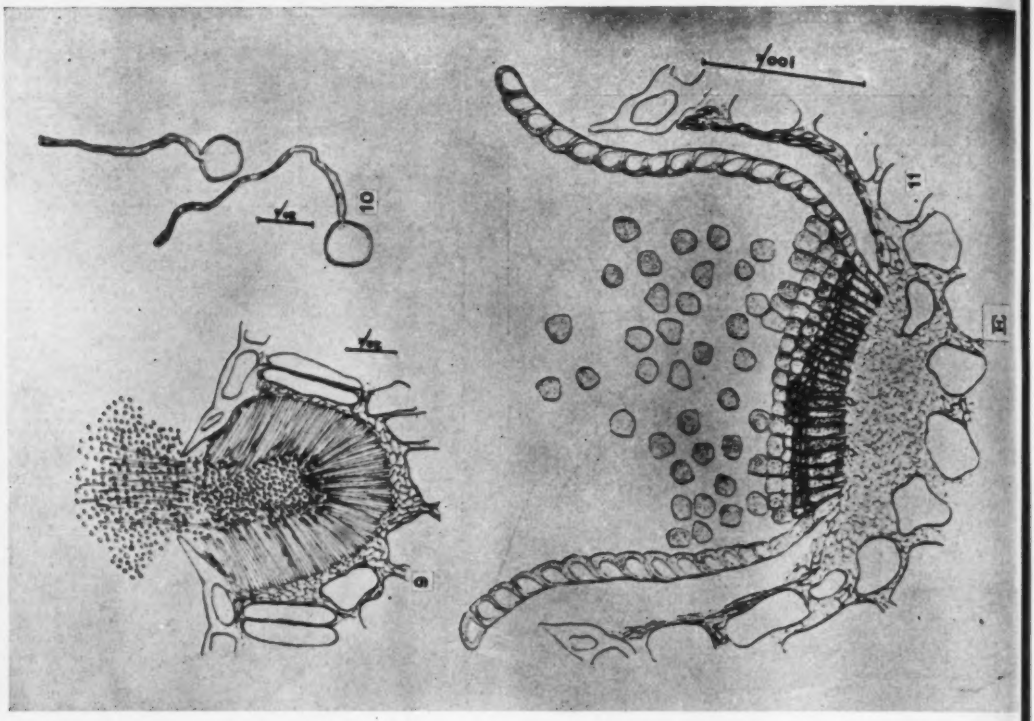
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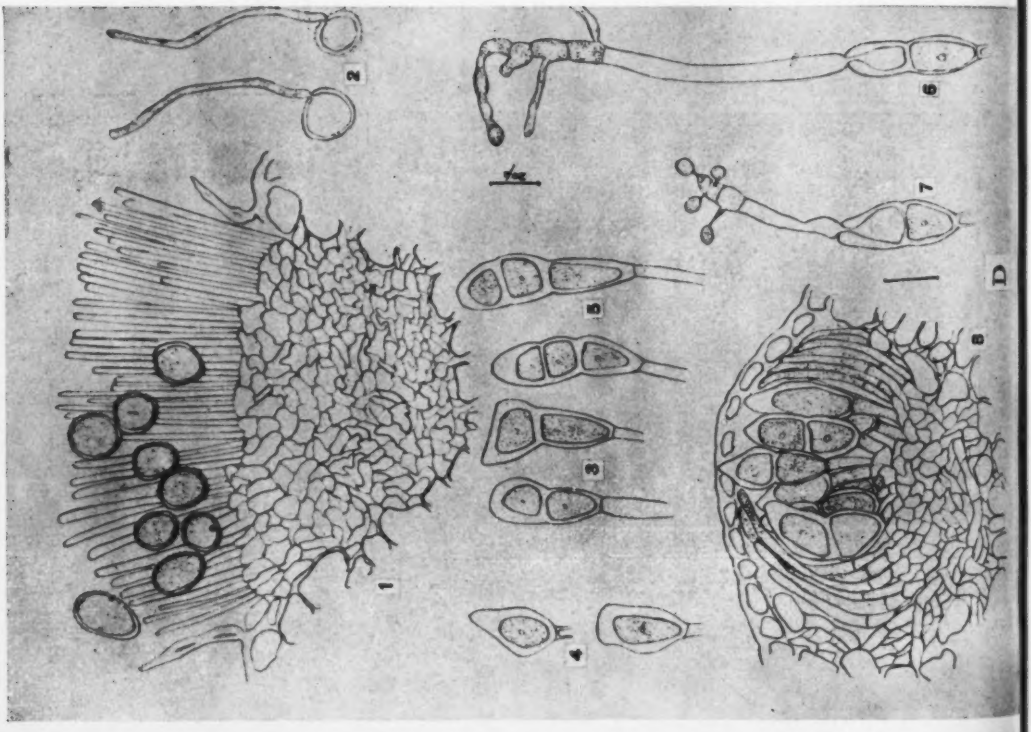
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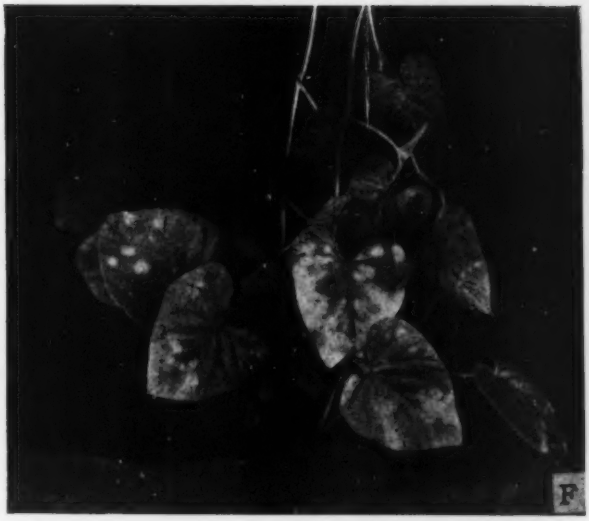
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pedicel persistent, light coloured, up to 68 μ; mesospores common, with rounded or flattened apex thickened up to 12 μ, 28–53 $\times$ 12–19 μ; three-celled teliospores also present.

Summary

The complete life-history of *P. romagnoliana* a common rust on *Cyperus rotundus* has been worked out. The rust is found to be heterœcious and *Ipomœa rumicifolia* and *I. pestigridis* are the alternate hosts. Aecia of more than one species of rusts are found to occur on this host genus.

Reference

Butler, E. J. and Bisby, G. R. .. "The Fungi of India," *Indian Coun. Agric. Res. Sci. Monogr.*, I, 1931.

Explanation of Plates

A. Symptoms of uredial and telial infection on *Cyperus rotundus.*

B & C. Aecidial formation on *I. pestigridis* and *I. rumicifolia* respectively.

D. 1. Section through the uredium; 2. Germinating urediospores; 3–5. Teliospores and mesospores; 6, 7. Germinating teliospores; 8. Section through the telium.

E. 9. Section through the pycnium; 10. Germinating æciospores; 11. Section through æcium.

F. *I. rumicifolia* inoculated with teliospores showing æcial formation.

THE BEARING OF CERTAIN RECENT CYTO-GENETICAL FINDINGS ON SUGARCANE BREEDING

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I. Introduction

As compared with other economic plants the improvement of sugarcane by breeding is of relatively recent origin. The discovery of the fertility of sugarcane seed was made by Soltwedel in Java in 1888 and independently by Harrison and Bovell in Barbados in 1889. Since then the work on sugarcane breeding has been vigorously pursued in Barbados, Java, Coimbatore and Hawaii, among the older Experiment Stations and at Canal Point, Florida and others among the later-established Stations.

While the main aim at the Sugarcane Breeding Stations is the evolving of high-yielding commercial canes with high sucrose content, many Experiment Stations have had to grapple with the necessity of breeding varieties resistant to one or the other of sugarcane diseases. The emphasis has also varied with regard to the utilisation of appropriate species of *Saccharum* and in certain cases, of different genera, in hybridisation to suit the needs of various cane-growing regions. Where cane develops under favourable conditions the noble (*S. officinarum*) and highly nobilised varieties have given good results as parents, while in less favourable areas

S. spontaneum and *S. barberi* have given more satisfaction. The sereh disease in Java led to the nobilisation of Chunnee and Glagah. The Coimbatore Station, for instance, has paid increasing attention to the utilisation of the wild *S. spontaneum* while the Station at Canal Point, Florida, has utilised *S. robustum*.

Much advance was registered in the breeding of sugarcane varieties by the utilisation of wide and distant genera in the breeding programme with a view to the enrichment of genetic material available to the breeder. Notable inter-generic crosses are those of sugarcane with *Sorghum* (Venkatraman and Thomas, 1932), (Raghavan, 1953 *a*) and Bamboo (Venkataraman, 1937). For the first time a " pure " *officinarum*, *viz*., Vellai, has been successfully crossed with Bamboo, one of them showing promise (Raghavan, 1952 *a*).

Sugarcane as a breeding material is fundamentally different from other commercial crops. It is a highly heterozygous polyploid complex with a large reserve of lethal genes, which especially in *S. officinarum* are resposible for there being practically no survivals in the selfed progeny. It is only the clonal propagation that has made it survive.

Arising mainly out of its genetical impurity and high allopolyploidy no prediction can be made as to the nature of the progeny to be expected in a cross. The high heterozygosity of the chromosomal material of both the parents renders odds against anticipating any desired combination. Consequently, repetition of well-known crosses has failed so far to give a variety identical with the original. For instance parental crosses for Co. 419 (POJ. 2878 × Co. 290) and Co. 421 (POJ. 2878 × Co. 285) have been repeatedly made for about 4 years. Though thousands of seedlings have been raised and studied there is not yet found an exact replica of either Co. 421 or Co. 419 (Raghavan, 1951 *a*, 1953).

Success in sugarcane breeding, therefore, depends to a large extent on the raising of a large population of seedlings. Thousands of seedlings are raised in bulk as well as semi-bulk scale crosses in the hope of getting some at least, with the desired combination of characters, and selections are made on the basis of suitability to different sets of environmental conditions. At Coimbatore the time interval from crossing upto the selection of Co. canes from the final test plots is about 4 years and the number of seedlings gets filtered down to about 25 from the lakhs of seedlings raised by hybridisation. These Co. canes are sent to the various State Stations for further extensive trials and only those canes are released that are found suitable for a particular set of environmental conditions.

II. UNPREDICTABILITY—INEVITABLE IN SUGARCANE BREEDING

Most of the interesting breeding peculiarities exhibited by sugarcane are traceable mainly to their being polyploid hybrid complexes (Parthasarathy, 1946, 1951 *b*, Raghavan, 1951 *a*, *b*, *c*, *d*). The selfed progeny shows a wide range of variation, which in the case of *S. officinarum* shows a very high rate of mortality owing to the presence of a large accumulation of recessive lethal genes in its genetical constitution. In addition, all sugarcanes are highly heterozygous and so all individuals resulting from selfing, varietal or species crosses are heterozygous also. The parental characters are found distributed among the progeny in an unpredictable manner. In some, the earliness of one parent may manifest itself; in others, the vigour of the parent; yet in a few, the high sucrose of one of the parents may be incorporated and it may also happen that in a very few a combination of most of the desirable characters of the two parents may be exhibited. It is obvious that such a seedling got out of mating of two highly heterozygous individuals cannot be re-synthesised. Fortunately for the sugarcane breeder such a form can be maintained through vegetative propagation.

For the reason that sugarcane is a polyploid complex, interspecific and even inter-generic hybrids are frequently fertile due to auto-syndesis, provided the two parental sets of chromosomes are compatible enough to exist side by side in the hybrid. Apart from species hybrids like *S. officinarum* × *S. spontaneum*, which are fertile, even inter-generic hybrids have shown fertility or partial fertility. The progeny from such fertile hybrids, though resembling one another, shows variation due to exchange of genes *among* the chromosomes of the respective parents, and not between them and the overall effect of the rearranged parental sets of chromosomes on the general configuration of the individual.

In sugarcane hybridisation no prediction can be made of what the chromosome number in a cross would be. In interspecific hybrids, *S. officinarum* × *S. spontaneum*, *S. officinarum* as the egg parent is known to contribute the diploid number. In reciprocal crosses *S. officinarum* contributes the unreduced number through its sperm (Raghavan, 1951 *a*, *d*). Nor is there gross identity between reciprocal crosses indicative of cytoplasmic inheritance (Raghavan, 1951 *b*, 1953 *b*, 1954). There is reason to suspect the presence of cytoplasmic inheritance as being an interaction between the genes and maternal cytoplasm. Non-reduction in the egg is due in some cases to fusion of the two lowermost megaspores in the linear tetrad (Narayanaswami, 1940) and in some other cases endomitosis of the lowermost tetrad (Bremer, 1949). Being a post-meiotic phenomenon, diploid partheno-

genetic derivatives exhibit wide variations, which would not be the case had the diploid egg come into being through suppression of meiosis. The way in which the male contributes the diploid number is variable. Many *S. officinarum* varieties as Zw. Cheribon, Chittan and Striped Mauritius, exhibit dispermy, while in the other species such as *S. spontaneum* or *S. robustum* diploid and polyploid pollen grains are common (Sundraraghavan, 1954).

In the evolution of some economic seedlings the egg is known to contribute a chromosome number which is neither its haploid nor its unreduced number. Sometimes, in the same cross, *S. officinarum* is capable of contributing haploid and diploid eggs, as well as eggs with an unexpected chromosome number through elimination of chromosomes. In *S. officinarum* ($2n = 80$) × *Sorghum* ($2n = 20$) crosses the hybrids showed $2n = 50$ ($n + n$) and 90 ($2n + n$) (Janaki Ammal; 1941; Raghavan, 1953 *a*) while *S. officinarum* × Bamboo ($2n = 72$) hybrids showed $2n = 116$ ($n + n$) and $2n = 86$ (unexpected egg + n, Raghavan, 1952 *a*). The contribution of eggs with 68 chromosomes on the part of the noble cane has been recorded in a few crosses, *S. officinarum* var. Lakhapur × *Sclerostachya* ($2n = 83$, Raghavan, 1952 *b*); *S. officinarum* var. Zw. Cheribon × Co. 285 ($2n = 112$) [Co. 453 ($68 + 56$) = 124, (Raghavan, 1951 *a*, *c*)]. In *S. officinarum* × *Sclerostachya* crosses all the four types, namely $n + n$ ($2n = 55$), $n + 2n$ ($2n = 70$), $2n + n$ ($2n = 95$) and unexpected egg + n ($2n = 60$) have been recorded (Govindaswami, 1948). The lowest number contributed by *S. officinarum* egg is 28, which is surprisingly enough, lower than the haploid number of *S. officinarum* (D. 74 × *S. spontaneum* Coimbatore = ($2n = 60$) (Parthasarathy, 1946, 1951 *a*).

III. Parthenogenesis and Chromosome Elimination, Major Factors in Sugarcane Breeding

In some of the pollen-sterile forms, parthenogenesis is common. So far no case of haploid parthenogenesis has been seen. The few cases that are known are diploid derivatives from unreduced eggs of pollen-sterile forms. Diploid parthenogenesis, being post-meiotic, the progeny shows wide variations due to recombination of genes as if the diploid egg has come into being through fertilisation. As such this phenomenon is of the utmost usefulness in sugarcane breeding. Many of the Co. canes are now known to be parthenogenetically derived. Table I gives the list of some Co. canes with their respective parents. Care has been taken to include in the list only such cases as show different chromosome numbers among the parents. In all these cases cited, the hybrid Co. canes show only the maternal number irrespective of the chromosome number of the paternal parent. Obviously

there is a very large number of other cases of parthenogenesis where there is numerical identity between the pistillate and the staminate parents.

Apart from diploid parthenogenesis, parthenogenesis preceded by elimination of chromosomes *en bloc* has been found to be of frequent occurrence. Important pollen-sterile canes, like Co. 421, Co. 602 and Co. 603, have shown this phenomenon. Co. 421 gives two types of seedlings parthenogenetically, one thick type with normal diploid number of $2n = 118$ and the other, thin type with $2n = 86$ chromosomes (Parthasarathy, 1946, 1951 *b*; Subramanian, 1946). In the same way Co. 602 gives rise to seedlings having $2n = 118$ and $2n = 96$ chromosomes, the former being diploid parthenogenetic derivative and the latter being a parthenogenetic derivative after chromosome elimination (Raghavan, 1954). As shown in Table II, Co. 803, Co. 804, P. 8388, P. 8415 and P' 1010/1 are all parthenogenetically derived preceded by chromosome elimination from Co. 603, the chromosome numbers being $2n = 108$, 108, 104, 86 and 104 respectively, while the chromosome number of Co. 603 itself is $2n = 118$ (Raghavan, 1953 *a*). For the first time this phenomenon has been observed in a " pure " species of *Saccharum*, *viz.*, *S. robustum*. When *S. robustum* with $2n = 84$ is selfed or crossed with distant genera, it is capable of giving rise to seedlings with $2n = 84$, 80 and also $2n = 62$ chromosomes (Sundraraghavan, 1954; Raghavan, 1954). So also Kassoer with $2n = 136$ when selfed gave a seedling with $2n = 112$ chromosomes (Raghavan, 1953 *b*).

Thus it seems abundantly clear that the phenomena of parthenogenesis and chromosome elimination play a more important role, than has been realised so far, in sugarcane breeding. It also appears that because of cytoplasmic inheritance and parthenogenesis the mother is bound to play a more important role than the staminate parent in sugarcane breeding.

IV. CYTOPLASMIC INHERITANCE—ITS ROLE IN SUGARCANE BREEDING

Since the finding of the existence of cytoplasmic inheritance in *Saccharum* (Raghavan, 1951 *b*, 1954), it has been made possible to anticipate to some extent the gross general appearance among the progeny of a cross, in the sense that there is a greater approximation to the mother than to the staminate parent. The non-expression of paternal characters in well-known inter-generic crosses with Bamboo, *Sorghum* and maize as male parent is now explicable on the basis of cytoplasmic inheritance. While the phenomenon has been more or less quite apparent as regards easily recognizable qualitative characters, it has been found that this holds good even in respect of quantitative characters like yield, purity, etc.

P. 8331 is a complicated hybrid now being increasingly used in hybridization for increasing richness in juice. Likewise Co. 605 gives uniformly good seedlings with high yield. Reciprocal crosses of the above parents were effected and population raised and studied. In the combination where P. 8331 was utilised as the female parent about 80% of the seedlings show high brix. (Raghavan, 1956). In the cross where Co. 605 is used as the female parent nearly 80% of the seedlings show high yield; the number of seedlings having high sucrose is comparatively low. This behaviour of the seedlings as regards these two characters is significantly different in the reciprocal crosses.

V. The Genetical Constitution of Some Economic Seedlings

In the genetical make-up of some of the economic seedlings selected year after year, the various cyto-genetical features of which an indication has been given, are found represented. Mention has already been made of the important role played by parthenogenesis and chromosome elimination in sugarcane breeding. Co. 205 and Co. 285 are the products of the fusion of the diploid egg of *S. officinarum* with haploid male gamete of *S. spontaneum* (Coimbatore). In the second nobilisation, generally, diploid eggs function. P' 449/1 is one such derivative being a cross between Chittan × Co. 205. It shows $2n = 136\,(80 + 56)$, diploid egg of *officinarum* having functioned. P' 451/1 is also a similar second nobilisation product. It is a cross between Chittan × A. 10 (Kaludai Boothan × *S. spontaneum*). The product of third nobilisation is through the fusion of haploid gametes. Such a derivative is POJ. 2878. At this Institute we have a number of parallel third nobilisation crosses. For instance P' 451/1 × Chittan shows 108—being a fusion product of haploid gametes. This cross has also been done reciprocally. It seems that higher polyploids in *S. officinarum* varieties are rare and the octoploid is the optimum level and higher ploidy is rather unfavourable and in many cases lethal (Raghavan, 1954). But in some of the seedlings of the second and third nobilisation crosses as in P' 451/1 and P' 749/1–3 even though the *officinarum* chromosomes are more than the octoploid level, the seedlings are vigorous. This is probably due to the fact that these seedlings have in addition to the large number of *S. officinarum* chromosomes, some *spontaneum* chromosomes also in their complement. P' 451/1 is having 120 *officinarum* chromosomes and 16 *spontaneum* chromosomes and in P' 749/1–3, out of 108 chromosomes, 100 chromosomes are from *officinarum* and 8 from *spontaneum*. This indicates that the presence of 8 chromosomes of *S. spontaneum* is enough to give requisite vigour to the seedlings (Raghavan, 1954). After all it is not the number but the right kind of chromosomes represented in the proper place, that matters most.

Further evidence in support of the above generalization is indicated by the findings of Liu and Li (1953). In tracing out the degree of mosaic resistance in noble canes, according to them, the hybrids maintain a good amount of tolerance to mosaic even though *S. spontaneum* chromosomes get reduced by slow degrees during successive stages of nobilisation. They point out that the presence of a few chromosomes of *S. spontaneum* (7–8) is sufficient for imparting resistance to mosaic.

In the early days of sugarcane breeding, *S. spontaneum* was extensively used because of the vigour and hardiness that it imparted to its progeny. Among such bispecies hybrids (having the genes of two forms in their constitution), mention may be made of Co. 205, Co. 285, Co. 421, Co. 432, Co. 453, Co. 605, etc., of which Co. 421 is doing well in certain parts of the tropics and the rest are sub-tropical. *S. Barberi* canes are also comparatively hardy and their juice quality is far superior to that of *S. spontaneum*, almost equalling that of the tropical canes. Hence it was thought desirable to build up tri-species hybrids (having the genes of three species in their constitution) using *Barberi* also as one of the parents. Such hybrids which have made a mark in North India are Co. 312, Co. 313, Co. 331, etc. In the tropics Co. 419, Co. 449, Co. 467, Co. 475 and Co. 527 are among those that have proved useful.

Among bispecies hybrids, which have not become commercial canes, but which could be employed as parents in breeding work, mention may be made of such forms as P. 3247 and P. 4626. By themselves they do not give high yields and therefore cannot be of commercial importance, but possess certain desirable individual characters which could be exploited through hybridisation. For instance P. 4626 is a derivative from the rich 'noble' cane Fiji B, whose soft-rindedness and good juice quality it has inherited. It is generally employed as a parent for evolving chewing canes as also rich canes of satisfactory yield. Among the recently released Co. canes, a number of them Co. 819 to Co. 831, have the 'blood' (= genes) of P. 4626 in them. Co. 475 and Co. 603 are promising canes having the genes of P. 3247 in their constitution.

From the tri-species hybrids also a number of parents have been isolated. One of the most outstanding is P. 2607, which though lacking in sucrose, imparts high tonnage and vigour to the seedlings. Co. 777 which was raised to Co. status recently is a derivative from this parent.

In addition to these three species of *Saccharum*, other genera have also been employed for building up good parents and economic types. The closely related genera *Narenga* and *Sclerostachya* were employed to impart resistance to water-logging, drought (hardiness) and diseases (Parthasarathy

and Venkatraman, 1942). A derivative from *Narenga* (P' 201/1) having *S. officinarum*, *S. spontaneum* and *Narenga* in its constitution is proving useful as a parent. The recently released Co. canes, Co. 827 to Co. 831 (Fiji B, G. C. × P' 201/1) have been derived from using this as a parent.

Another good parent is P' 63/32 (Janaki Ammal, 1941) which has in its composition not only genes from the two species of *Saccharum*, *viz.*, *S. officinarum* and *S. spontaneum*, but also those of the genus *Imperata*. This usually imparts high juice quality, as also earliness to its seedlings. Co. 644 is one such derivative. Recently a number of seedlings having this as one of the parents have been raised to the status of Co. canes. The cross Co. 603 × P'63/32 has given a number of forms, *viz.*, Co. 803 to Co. 814, with different useful characters distributed among them.

SUMMARY

In sugarcane breeding the appropriate species of *Saccharum* and in certain cases of different genera, have been utilised to suit the needs of the various cane-growing regions. The Coimbatore Station for instance has paid increasing attention to the utilisation of *S. spontaneum* while the Station at Canal Point, Florida, has utilised *S. robustum*. The *Spontaneum* Expedition Scheme is in furtherance of this objective.

The cyto-genetical basis for the (seemingly capricious) breeding behaviour of sugarcane, has been briefly indicated. Parthenogenesis and chromosome elimination coupled with cytoplasmic inheritance, seems to indicate the greater importance which has to be bestowed upon the pistillate parent in sugarcane breeding. The constitution of some of the important economic seedlings, has been explained from this point of view.

TABLE I

Diploid Parthenogenesis

Sl. No.	Cross	Hybrid
1	Co. 421 ($2n = 118$) × *S. officinarum* var. Striped Mauritius ($2n = 80$)	Co. 463 ($2n = 118$)
2	Co. 421 × *S. officinarum* var. Poovan ($2n = 80$)	Co. 602 ($2n = 118$)
3	Co. 421 × Co. 453 ($2n = 124$)	Co. 656 ($2n = 118$)
4	Co. 602 ($2n = 118$) × Coix, *Sorghum*, Teosinte etc. (all $2n = 20$)	$2n = 118$
5	Co. 603 ($2n = 118$) × E.K. 28 ($2n = 80$) ..	Co. 817 ($2n = 118$)
6	Co. 603 × P' 63/32 ($2n = 120$–24) ..	Co. 909, Co. 910, Co. 811 (all $2n = 118$)
7	POJ. 2725 ($2n = 106$–08) × *Sorghum durra* ($2n = 118$)	Co. 356 ($2n = 106$–108)
8	POJ. 2725 × B. 3412 ($2n = 80$)	Co. 407 ($2n = 106$–108)
9	POJ. 2725 × *S. spontaneum* Coimbatore ($2n = 64$)	Co. 455 ($2n = 106$–108)

TABLE II

Parthenogenesis Preceded by Elimination of Chromosomes en bloc

Sl. No.	Cross	Hybrid
1	Co. 421 ($2n = 118$) self	P'212/8 ($2n = 86$)
2	Co. 602 ($2n = 118$) × Coix ($2n = 20$) ..	Seedling No. 18 ($2n = 96$)
3	Co. 602 × Maize ($2n = 20$)	Seedling No. 20 ($2n = 96$)
4	Co. 603 ($2n = 118$) × P'63/32 ($2n = 120$–24)	Co. 803 ($2n = 108$)
5	Co. 603 ($2n = 118$) × P' 63/32 ($2n = 120$–24)	Co. 804 ($2n = 108$)
6	Co. 603 × Co. 449 ($2n = 118$)	P. 8388 ($2n = 104$)
7	Co. 603 × Co. 449 ($2n = 118$)	P. 8415 ($2n = 84$–86)
8	Co. 603 self	P' 1010/1 ($2n = 104$)
9	*S. robustum* self ($2n = 84$)	P' 868/1 ($2n = 80$)
10	*S. robustum* ($2n = 84$) × Coix ($2n = 20$) ..	P'875/1 ($2n = 62$)
11	*S. robustum* × *Sorghum* ($2n = 20$)	P'873/2 ($2n = 62$)
12	Kassoer ($2n = 136$) Self	P' ($2n = 112$)

REFERENCES

Bremer, G. .. "Increase in chromosome number in species hybrids of *Saccharum*," *Proc. 8th Int. Cong. Genet., Suppl. to Hereditas*, Lund, 1949, 541-42.

Govindaswami, S. .. *Thesis* submitted to the Univ. of Madras for the Degree of M.Sc., 1948.

Janaki Ammal, E. K. .. "Intergeneric hybrids of *Saccharum*," *Jour. Hered.*, 1941, 217.

Liu, H. P. and Li, H. W. .. "Studies on the sugarcane mosaic virus in Taiwan," *Taiwan Sug. Expt. Stn.*, 1953, No. 10.

Narayanaswami, S. .. "Megasporogenesis and the origin of triploidy in *Saccharum*," *Ind. Jour. Agri. Sci.*, 1940, **10**, 534–51.

Parthasarathy, N. .. "The probable origin of North Indian Sugarcanes," *M. O. P. Iyengar Commem. Vol., Jour. Ind. Bot. Soc.*, 1946, 133–50.

———— .. "Some cytogenetical aspects of the origin of sugarcane," *Ind. Jour. Genet. and Pl. Breeding*, 1951 *a*, **2**, 63-66.

———— .. "Chromosome elimination in *Saccharum*," *Nature*, 1951 *b*, **168**, 383.

——— and Venkatraman, T. S. "Chromosome counts in sugarcane and its hybrids," *Curr. Sci.*, 1942, **11**, 194–95.

Raghavan, T. S. .. "The Sugarcanes in India: Some cytogenetic considerations," *Jour. Hered.*, 1951 *a*, **42** (4), 199-206.

———— .. "Cytoplasmic inheritance in *Saccharum*," *Curr. Sci.*, 1951 *b*, **20**, 138.

———— .. "Some aspects of recent cytogenetical work in sugarcane," *Proc. I Bienn. Conf. Sugarcane Res. Workers*, Coimbatore, 1951 *c*.

———— .. "Cytogenetics of sugarcane," *Ind. Jour. Agric. Sci.*, 1951 *d*, **22**, 93.

Raghavan, T. S. .. "Sugarcane × Bamboo hybrids," *Nature*, 1952 *a*, **170**, 329.

———— .. *Annual Report of Sec. Cane-Breeding Officer*, 1952 *b*.

———— .. *Ibid.*, 1953*a*.

———— .. "Some cytogenetical features and breeding behaviour of a *Saccharum* spont. derivative," *Proc. I.S.S.C.T., 8th Cong.*, Barbados, 1953 *b*, 475–85.

———— .. "Cytogenetics in relation to sugarcane breeding," *Cytologia*, 1954, **19**, 133–43.

———— .. "Sugarcane as a material for Genetical studies," 1956 *Proc. I.S.S.C.T. 9th Congress*, India.

Subramaniam, C. L. .. *Thesis* submitted to the Univ. of Madras for the Degree of M.Sc., 1946.

Sundraraghavan, R. .. "Morphological and cytogenetical features in *S. robustum* and its interspecific and inter-generic hybrids," *Thesis* submitted to the Univ. of Madras for M.Sc. Degree, 1954.

Venkatraman, T. S. and Thomas, R. "Sugarcane × *Sorghum* hybrids: Part I. General outline and early characters," *Ind. Jour. Agric. Sci.*, 1932, **2**, 19-27.

Venkatraman, T. S. .. "Sugarcane × Bamboo hybrids," *ibid.*, 1937, **7**, 513.

A COMPARATIVE CHEMICAL STUDY OF FLUE-CURING AND SHADE-CURING OF VIRGINIA TOBACCO

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Introduction

The cigarette and cigar types of tobacco are cured by two entirely different methods, the former by flue-curing and the latter by air-curing. Also the cigarette type of leaf is rich in carbohydrates and poor in nitrogenous constituents while the reverse is true of the cigar type. The chemical changes that occur under the two systems of curing using the requisite type of leaf have been thoroughly investigated by a number of workers. However, there is great paucity of information on the chemical changes that occur when the same leaf is subjected to the two modes of curing. The work of Askew *et al.* (1954), which is later than the one under report, is the most relevant to the present studies. While Askew *et al.* (*Loc. cit.*) held the leaf in their Experiment 1–A, which was being air-cured, under atmospheric conditions in the dark, the leaf in the present studies was held under atmospheric conditions in a shade. This was done as some cigar tobaccos in India (Lanka tobacco) are cured under shade. Also the air-curing experiment carried out by Askew and co-workers had been terminated after 64¾ hours while the present studies were carried on for 123 hours. Because of these differences in treatment which in their turn caused certain differences in chemical changes observed by Askew *et al.* (*Loc. cit.*) and the author, it is thought necessary to report on the findings in the present investigation.

The purpose of the present paper is to see whether the general run of chemical processes in a flue-cured type of leaf (Harrison's Special) is similar both in air-curing and in flue-curing or whether the changes described by Vickery *et al.* (1931 and 1953) are evidenced in a flue-cured leaf when it is air-cured. This knowledge is necessary to understand the biochemistry of tobacco leaf curing.

Experimental

Material and Methods.—Harrison's Special, a well established flue-cured variety in India, was employed in these studies. It is a high yielder,

possesses a fine silky texture and cures to a bright lemon yellow colour. It is extensively grown in the Guntur, Kistna and East Godavari districts of the Andhra State.

The leaf material employed in this study was taken from the experimental plots of the Tobacco Research Sub-station, Guntur, in the year 1947. The plots were treated with 3 tons of farmyard manure per acre. The leaf was picked from the plant at the right stage of maturity and second picking leaves were employed. They were divided into the required number of lots by random sampling. Their green weight was determined and they were strung separately to sticks which were suitably labelled. Half the number of lots were shade-cured and the other half flue-cured. They were removed from the flue-curing barn and also shade-curing shed at the end of 40 hours, 55 hours and 123 hours. These corresponded to the end of yellowing, end of fixing and end of curing in flue-curing. The samples were immediately weighed and preserved by steaming and drying in a current of air at 65° C. as recommended by Loomis and Schull (1937).

The initial sample was collected in quadruplicate to afford a measure of sampling errors and the others in duplicate. The averages of the results are presented in this paper.

The constituents determined and the analytical methods employed were the same as described by the author in a previous publication (Sastry, 1953). In addition amino nitrogen was determined by Sorensen Method (A.O.A.C., 1950, p. 365). No nitrates could be found in any of the samples.

The data are expressed in terms of 100 grams of the initial weight of leaves.

Sampling and analytical errors.—The initial sample was collected in quadruplicate and analysed for the various constituents, mentioned above, to afford a measure of the sampling and analytical errors. The mean values and standard deviation are presented in the various tables and any variation from the mean value which is twice the standard deviation is taken as significant and this is used in the interpretation of the present data.

Results

Physical changes in the leaf.—Visual examination of the leaf was carried out as soon as it was taken out from the flue-curing barn or air-curing shed. Though no figures have been collected for this purpose it was observed that while at the end of 40 hours, the leaf in flue-curing had completely yellowed, the leaf under shade-curing was not yellow even at the margins and tips,

Even at the end of 123 hours which was the time taken for the entire flue-curing process, the leaf in shade-curing was only about 75 per cent. yellow.

Chemical Changes : Water and Solids.—Changes in the weight of leaves, water and solids are presented in Table I.

TABLE I

Distribution of water and solids during flue-curing and shade-curing expressed as grams per 100 grams fresh leaf

(Expressed as grams per 100 grams fresh leaf)

	Shade-curing				Flue-curing		
	0 hr.	40 hr.	55 hr.	123 hr.	End of yellowing 40 hr.	End of fixing 35 hr.	End of curing 123 hr.
1 Weight of leaves	100·00	67·00	57·85	37·49	75·90	58·68	18·37
2 Water ..	78·99	48·12	40·41	20·60	57·40	40·95	0·64
3 Total solids ..	21·01 ±0·35	18·88	17·44	16·89	18·50	17·73	17·73
4 Inorganic solids	3·23 ±0·1	3·01	2·88	2·95	2·99	3·05	3·00
5 Organic solids..	17·78	15·87	14·56	13·94	15·51	14·68	14·73

Taking into consideration the first 40 hours, when the leaf was alive in both the methods of curing, it is to be observed the weight of leaf and its moisture content decreased more sharply in shade-curing than in flue-curing. Conditions of curing employed in these studies are shown in Figs. 1 and 2 and the average conditions of curing during the first 40 hours are shown in Table II.

TABLE II

Conditions of curing during the first 40 hours

	Average temperature °F.	Average relative humidity %	Evaporative power of the air
Flue-curing	93·3	84·1	0·375
Shade-curing	77·5	73·5	0·387

Higher humidity and temperature prevailed during flue-curing than in shade-curing. However, the evaporative power of the air was almost the

Temperature
160
150
140
130
120
110
100
90
80
70
Flue-curing
Shade-curing
10 20 30 40 50 60 70 80 90 100 110 120 130
HOURS

FIG. 1

Relative Humidity %
100
90
80
70
60
50
40
30
Flue-curing
Shade-curing
10 20 30 40 50 60 70 80 90 100 110 120 130
HOURS

FIG. 2

same in the two modes of curing during this period. The greater loss of water during shade-curing therefore appears to be due to the greater exposure of the leaf which is in shade as compared with that in the flue-curing barn.

The slight changes in inorganic solids noted, are probably due to experimental errors and the imperfect method of determination. The changes in solids could therefore be taken as due entirely to the organic solids. During the first 40 hours there was more loss of organic solids under flue-curing than in shade-curing while the loss in weight and moisture content of leaf was less than in shade-curing.

Also there were differences in the rate of loss of solids in the two methods of curing under study. Under shade-curing though there were variations in the rate of loss of solids, the loss was continuous throughout the period. In flue-curing, on the other hand, the rate of loss of solids was rapid during yellowing and fixing and during drying there was no loss. This becomes explicable when we note that in shade-curing the leaf was alive during the entire period of study, while in flue-curing the leaf was completely dead at the end of fixing.

Carbohydrates.—The changes in carbohydrate constituents are shown in Table III.

TABLE III

Distribution of carbohydrates during flue-curing and shade-curing

(Expressed as grams per 100 grams fresh leaf)

	Shade-curing				Flue-curing		
	0 hr.	40 hr.	55 hr.	123 hr.	End of yellowing 40 hr.	End of fixing 55 hr.	End of curing 123 hr.
1 Starch as glucose	3·26 ±0·166	1·46	0·68	0·06	1·03	0·40	0·19
2 Reducing sugars as glucose	0·54 ±0·095	1·18	1·58	1·80	1·47	1·77	1·87
3 Sucrose as glucose	0·0	0·27	0·28	0·07	0·16	0·03	0·27
4 Total carbohydrate (1+2+3)	3·80	2·91	2·54	1·93	2·66	2·20	2·33

Starch was hydrolysed during the first 55 hours in both the curings though during flue-curing it was more rapid than in shade-curing. Between 55 and 123 hours the rate of hydrolysis decreased, though during shade-curing more starch was hydrolysed during this period than in flue-curing.

As the hydrolysis of starch progressed reducing sugars increased rapidly, though during the early phases, the rate of their formation was more in flue-curing than in shade-curing.

The formation of sucrose does not appear to be of much importance in these leaves, though the data suggest that small quantities of sucrose which were formed during the first 55 hours of shade-curing were used up by the leaf during the later stages. In flue-curing, on the other hand, a little sucrose appears to be synthesised during the drying stage.

Nitrogenous constituents.—Nitrogenous constituents are presented in Table IV.

TABLE IV

Distribution of nitrogenous constituents during flue-curing and shade-curing

(Expressed as milligrams per 100 grams fresh leaf)

	Shade-curing				Flue-curing		
	0 hr.	40 hr.	55 hr.	123 hr.	End of yellowing 40 hr.	End of fixing 55 hr.	End of curing 123 hr.
1 Total N ..	420±8	418	368	381	397	402	411
2 Protein N ..	322	245	185	190	235	209	203
3 Soluble N ..	98±14	173	183	191	162	193	208
4 Amino N ..	3±1	9	17	21	19	22	21
5 Ammonia N ..	1·5±0·3	2·3	2·5	5·3	2·8	4·6	5·2
6 Amide N×2 ..	11·0±2	11·0	12·6	15·0	11·2	23·2	20·8
7 Nicotine N ..	23±0·6	23	23	25	24	25	26
8 Rest N ..	54	122	128	124·7	105	118·2	133

The changes in total nitrogen were different under the two modes of curing. In shade-curing as well as in flue-curing there was no change in the total nitrogen content of the leaf during the first 40 hours. The slight decrease observed in flue-curing is not much beyond the experimental errors, indicated in Table IV. Later on this constituent decreased sharply in shade-curing resulting in a loss of 9·21 per cent. of that originally present in the leaf while in flue-curing it remained more or less at the same level.

Protein hydrolysis occurred at almost the same rate during the first 40 hours in both the curings. While this rate was maintained between 40 to 55 hours in flue-curing it increased sharply during this period under shade-

curing. It is of interest to note that considerable amounts of nitrogen were lost from the leaves during the same period in shade-curing.

With the hydrolysis of proteins, soluble nitrogen and amino nitrogen (as determined by formal titration) increased. The data indicate that during the first 40 hours, for almost similar amounts of protein hydrolysed, only 6 milligrams of amino nitrogen were formed during shade-curing, while 16 milligrams were formed during flue-curing. During the later stages the rate of formation of amino nitrogen was considerably slowed down in flue-curing. In shade-curing on the other hand the production of amino nitrogen continued throughout the period of study.

Ammonia increased steadily during the entire period of study in shade-curing so that at the end the leaf contained about 3½ times as much ammonia as at the beginning. This, however, did not result in any significant amide formation. In flue-curing also the amount of ammonia was doubled at the end of yellowing, but this did not result in any significant amide formation. These observations agree with those of Vickery *et al.* (1940) who found that though ammonia increased to high concentrations in tobacco plants sown in sand culture, amides did not increase with ammonia, thereby showing that tobacco does not respond to ammonia accumulation by amide synthesis.

The data does not show any significant changes in the nicotine nitrogen content during the two processes of curing. A similar result was obtained by Askew *et al.* (1954).

DISCUSSION

Though there was reference to a trial by U. S. Department of Agriculture (1941) in which comparable leaves of a flue-cured type were air-dried and flue-cured, analytical data for it do not appear to have been published. The only other available reference to this type of work is that of Askew *et al.* (1954). The leaves that were air-cured in this work were held under atmospheric conditions in the dark, while the leaves in the present study were subjected to atmospheric conditions in a shade. This appears to have made some difference as may be seen from the water and total solids data.

In Askew's leaves there was a slightly greater loss in moisture in air-curing than in flue-curing (about 1% more). In the present set of leaves the leaves in shade-curing lost about 9% more water during the first 40 hours than those in the flue-curing barn, though the evaporative power of the air was about the same in both the systems of curing (Table III). This may be because of the greater exposure of the leaf in the present study as compared with Askew's.

As regards solids there was a greater loss of solids in flue-curing than in shade-curing during the first 40 hours. Similar results were obtained by Askew *et al.* (*Loc. cit.*). The average conditions of temperature and humidity during the first 40 hours in the two systems of curing have been indicated in Table II. It may be seen that higher temperature and humidity prevailed during flue-curing. In Askew's work also the temperature in the flue-curing barn was about 20° F. higher than in air-curing in the early phases. According to Garner *et al.* (1914) the rate of curing is proportional to temperature and the rate of increase in speed of reactions tends to obey the law of Van't Hoff, according to which the speed of ordinary chemical reactions is approximately doubled for each increase of 10° C., provided the optimum temperature for enzyme action is not much exceeded. The greater loss of solids in flue-curing may in part, at least, be due to the higher temperature prevailing in the flue-curing barn. The following results recalculated from Johnson *et al.* (1931) also show similar trends. From their data the dry weights of primed leaves cured under different temperature and humidities were recalculated and presented in Table V.

TABLE V

Temperature °F.	R.H. %	Daily evaporation c.c.	Green weight of primed leaves	Dry weight after 21 days cure	Dry weight % green weight
59	62	11·9	690·7	60·3	8·73
77	81	11·0	660·3	55·3	8·38
95	92	14·4	651·3	50·3	7·72

At higher temperature and higher humidity, even when the evaporative power of the air was almost the same, there was more loss of solids in the same leaf.

The carbohydrate balance during the different stages of curing is given in Table VI.

During the first 40 hours when the leaf was alive in the two processes of curing the loss of carbohydrates expressed as a percentage of the loss of organic solids was 50·33% in flue-curing and 46·60% in shade-curing. During the next 15 hours loss of carbohydrates as a percentage of the loss

TABLE VI

Carbohydrate balance during flue-curing and shade-curing

(Expressed as grams per 100 grams fresh leaf)

	0–40 Hours		40–55 Hours		55–123 Hours	
	Flue-curing	Shade-curing	Flue-curing	Shade-curing	Flue-curing	Shade-curing
1. Loss in organic solids	2·27	1·91	0·83	1·31	−0·05	0·62
2. Loss in carbohydrates	1·14	0·89	0·46	0·37	−0·13	0·61
3. Carbohydrates % organic solids	50·33	46·60	55·43	28·24		

of organic solids was only 28·24 under shade-curing, while it was 55·43% in flue-curing. These figures suggest that during shade-curing where the reactions were slower more of other constituents than carbohydrates were contributing to respiratory losses, than in flue-curing.

It is of great scientific interest to see if the changes reported by Vickery *et al.* (*Loc. cit.*) took place in the present study also. According to Askew *et al.* (*Loc. cit.*) the same reactions will proceed in the flue-cured leaf in air-curing as in flue-curing, except that they may be hastened owing to higher temperature in the later case. There was little or no loss of total nitrogen in their work under both the systems of curing. In the present study the changes in total nitrogen observed during flue-curing were not much beyond the experimental errors. This corroborates the previous findings of the author (1953). However, under shade-curing there was a distinct loss of total nitrogen from the leaves between 40–55 hours amounting to about 10%. This finding is in confirmity with those of Vickery *et al.* (1931) who reported a loss of 15% nitrogen during air-curing of Connecticut shade-grown tobacco. In a later publication (1953) they reported a loss of 6% nitrogen during the same process. The reported failure of Askew *et al.* (*Loc. cit.*) to note any loss of nitrogen in their air-curing experiment is presumably because they terminated their experiment after 64¾ hours. Had they continued the experiment longer they, probably, would have found out similar losses. Amino nitrogen, amides and ammonia increased steadily throughout the shade-curing process. However, the present studies unfortunately have not been conducted beyond 123 hours, otherwise probably the maxima for amino

acids, amides and ammonia described by Vickery *et al.* (*Loc. cit.*) would have been observed here also.

The present studies not only confirm the findings of Askew *et al.* (*Loc. cit.*)—that in the same leaf under flue-curing where higher temperatures prevail, the reactions are hastened as compared with shade-curing, but also tend to show that even in a flue-cured leaf some of the reactions characteristic of air-curing occur when it is subjected to air-curing.

Summary and Conclusions

In order to study the biochemical peculiarities of flue-curing and air-curing a flue-cured type of leaf was subjected to both the systems of curing.

During the first 40 hours which corresponded to the yellowing stage in flue-curing, the leaf was at a higher temperature and humidity in flue-curing than in shade-curing.

This resulted in hastening of chemical reactions in flue-curing as compared to shade-curing. The leaf was completely yellow at the end of 40 hours in flue-curing, while it was only 75% yellow at the end of 123 hours in shade-curing. Also there was greater hydrolysis of starch, more production of sugars and finally greater loss of solids in flue-curing than in shade-curing.

The contribution of carbohydrates to respiratory losses in the early stages was more in flue-curing than in shade-curing, showing thereby that more of other constituents than carbohydrates contributed to these losses in shade-curing.

Probably because of this there was considerable loss of nitrogen under shade-curing while the changes in this constituent during flue-curing were insignificant.

The present study thus tends to show two facts.

1. Due to higher temperature in flue-curing the chemical reactions are hastened as compared with shade-curing.

2. Even in flue-cured leaf, some reactions characteristic of air-curing occur, if it is subjected to air-curing.

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REFERENCES

A.O.A.C. .. 1950, 365.

Askew, H. O., Monk, R. J., Joyce, H. and Ward, G. *N.Z. Jour. Sci. Tech.*, 1954, **35B**, 344.

Garner, W. W., Bacon, C. W., and Foubert, C. L. *U.S.D.A. Bull.*, 1914, **79.**

Johnson, J. and Ogden, W. B. .. *Wis. Agric. Expt. Stat. Res. Bull.*, 1931, **110.**

Loomis, W. E. and Schull, C. A. *Methods in Plant Physiol.*, 1937.

Sastry, A. S. .. *Proc. Ind. Acad. Sci.*, 1953, **38**, 125.

U.S.D.A. .. *Annual Rpt. Bur. Pl. Ind.*, 1941, 35.

Vickery, H. B. and Pucker, G. W. *Conn. Agr. Expt. Stat. Bull.*, 1931, **324.**

———, ———, Wakeman, A. J. and Leavenworth, C. S. *Ibid.*, 1940, **442.**

——— and Meiss, A. N. .. *Ibid.*, 1953, **569.**

STUDIES ON THE NUTRITION OF URBAN POPULATION GROUPS

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Introduction

The areas of the world in which Kwashiorkar is prevalent are very similar to, although not identical with, the areas in which cirrhosis and primary carcinoma of the adult human liver have a high incidence. Hance (1928), Rao (1934) and Huges (1935) have recorded a high incidence of cirrhosis of the livers among the Indians of the poor class. Menon and Annamalai (1935) have reported that alcohol could not be implicated as a cause and have suggested that dietary deficiency may be an important ætiological factor. From a study of the available dietary history of patients Fernando, *et al.* (1948) have reported that fatty infiltration and cirrhosis of the liver are associated with a lack of lipotropic factors in the diet. Gilbert and Gilman (1944) have recorded that the fatty liver was the commonest pathalogical finding in malnourished African infants and to a lesser extent in adults, suffering from acute nutritional breakdown. Mc Farlane and Brandy (1945) Platt (1945) and Witts (1947) have reported that infantile cirrhosis of the liver was particularly common among children of mothers who had to support themselves and their children by working in urban areas or plantations. Breast feeding in such cases was scanty and infant diets consisted of arrow-root, cereal gruel, or even sugar and water. The staple of the diet has often been associated with the nature of the nutritional syndromes appearing in any region. In all the regions with a great prevalence of cirrhosis of the liver, the bulky vegetarian diet has been found to be excessively rich in carbohydrates. An attractive hypothesis that the gross hepatic damage of infantile kwashiorkor is the starting point of a fibrosis which proceeds insidiously throughout adoloscence and presents itself clinically in the third and fourth decades of life as a " nutritional cirrhosis " sometimes complicated by primary carcinoma of the liver, has been suggested by Brock (1954) to explain the areas of correspondence of Kwashiorkar, Cirrhosis of the liver and primary carcinoma of the liver. In discussing the pathology of

the liver in Kwashiorkar Davies (1952) states that "if the child survives either with or without treatment the fat tends to disappear slowly from the periphery leaving behind a stellate fibrosis and round cell infiltration on the portal triads. Treatment with protein leads to rapid fat reduction and a flooding of the sinusoids with lymphocytes, but does not affect the fibrosis and cell accumulation. They may persist for many years, if not for life. Why they should be so persistent when in other conditions quite well marked fibrosis of the liver can disappear is a mystery; but it suggests that there is a continuing liver damage of some sort." What then is this "continuing liver damage of some sort?" Is it continuing protein malnutrition or do other cirrogenetic factors play any part? Viruses, tropical parasites, alcohol, hepatotoxic alkaloids and excessive iron intake are the possible or probable cirrogenetic agents that could play a part. There has not been any conclusive evidence about the part played by these cirrogenetic agents and the concept that malnutrition and more particularly protein malnutrition is important in the genesis of human liver cirrhosis emerges. Study of the epidemiology of the disease in relation to the prevailing dietary patterns and other possible ætiological mechanisms should reveal valuable information.

Wahi (1949) has successfully produced cirrhosis of the liver in the albino rat by feeding a diet which is claimed by the author to be the nearest approach to the diet of vegetarian Hindus. But his diet has been criticised by Vijayaraghavan and Patwardan (1952) as synthetic and so not resembling the natural one. The latter have fed a natural diet based on the diet survey of Aykroid and Krishnan (1936) to rats continuously for a long time. but this diet could not produce cirrhosis of the liver. The dietary regimen used by Vijayaraghavan and Patwardan consisted of the following constituents:

Half milled Rice	.. ..	71·5 parts
Milk	.. .. ..	7·1 „
Pulses	.. .. ..	3·6 „
Amaranth	.. ..	7·1 „
Brinjal	.. .. ..	7·1 „
Gingelly Oil	.. ..	1·8 „
Common Salt	.. ..	1·8 „

In the dry districts like North Arcot the dietary pattern is different. Availability of milk and green vegetables is poor. Also this region is noted for its great incidence of cirrhosis of the liver. The number of patients with cirrhosis of the liver admitted as inpatients in the Christian Medical College Hospital, was 91 for the year 1954. Outpatients records showed an attendance of 106 during the same period, though the actual number should be

TABLE I

Classification of the Families Surveyed

Group No.	Type	Occupation	Vegetarian or Non-vegetarian	No. of families	No. of individual concerned	Total income of the group (in Rs.)	Average income per family	Average income *per capita*
1	Well-to-do (Hindu) ..	Officials and Business Men	Vegetarian	31	220	6580	212·3	29·9
2	Do. ..	Do.	Non-vegetarian	26	274	4050	156·0	24·4
3	Lower Middle Class (Hindu)	Mostly Officials	Vegetarian	40	230	3000	75·0	13·04
4	Do. ..	Mostly Business Men	Non-vegetarian	20	126	2070	103·5	16·4
5	Do. (Christian)	Officials ..	Do.	20	110	1956	97·5	17·5
6	Do. (Muslim)	Petty Traders ..	Do.	24	159	2040	85·0	13·0
7	Poor (Hindu) ..	Skilled labour	Do.	144	800	5888	40·9	7·36
8	Do. ..	Artisans Unskilled labour ..	Do.	162	816	8370	20·8	4·12
9	Hindu ..	Conservancy workers	Do.	22	88	1360	62·0	15·5
10	Poor (Christian) ..	Unskilled labour	Do.	12	64	460	35·0	7·2
11	Do. (Muslim) ..	Do.	Do.	6	28	200	33·3	7·0
			Total ..	507	2615	..	..	11·04

much greater on account of the large numbers in the outpatient departments who could not be attended to. Many of these patients were from the town and outlying areas. The Municipal death register records 51 deaths due to cirrhosis of the liver during the same period. Here again the actual number should be much greater. The object of this investigation is to make a survey of the diet consumed in these parts in order to obtain the pattern of diet. When once these dietary patterns are established by a survey, animal work can be done, to assess the type of the liver injury that it could produce.

METHODS

The diet survey was made by the "family method" with a seven-day period of observation (Canadian Studies on Nutrition, 1941) during the months of April-July. The families were classified into groups based on their religion, occupation and monthly income. A total of 507 families comprising 2,815 individuals were surveyed. The classification of the families is given in Table I. A separate form was used for each family and the foods were grouped under various headings; cereals, pulses, vegetables, oil, milk products, meat, fish and eggs, condiments, sugar and other foods. The net income per family was also ascertained. A record of the age and sex of all the members of the family partaking the food, as also those of guests when present was kept.

In calculating the consumption units perfamily it was found necessary to slightly modify the international scale of family coefficients (League of Nations, 1932) in the light of experience and actual observations to suit Indian conditions. The actual scales adopted are given in Table II.

TABLE II

Scale of Family Coefficients used in the Calculation of Consumption Units

(Figures represent the intake at different ages in fraction of adult male intake)

Age	Male	Female
0– 2	0·2	0·2
2– 3	0·3	0·3
4– 5	0·4	0·4
6– 7	0·5	0·5
8– 9	0·6	0·6
10–11	0·7	0·7
12–13	0·8	0·8
14–49	1·0	0·8
50–59	0·8	0·8
Over 60	0·5	0·5

The actual consumption of food stuffs by the various groups are given in Table III. The results of earlier investigations along with recommended intakes are given in Table IV for comparison.

TABLE III

Consumption of Food in Ounces—Average Daily Intake per Consumption Unit

Article of Diets	Well-to-do (Hindu) vegetarian (1)	Well-to-do (Hindu) non-vegetarian (2)	Lower middle class (Hindu) vegetarian (3)	Lower middle class (Hindu) non-vegetarian (4)	Lower middle class (Christian non-vegetarian (5)
Total cereals	15·8	16·0	15·2	15·9	15·0
Rice (Polished)	14·0	15·6	13·8	13·0	12·2
Wheat (flour)	1·8	0·4	0·5	..	1·5
Ragi	..	..	0·9	2·9	1·3
Cholam	..	..	..	..	..
Total Pulses	2·55	1·9	2·53	1·94	1·25
Red Gram (Dhal Arhar)	1·5	1·0	1·4	1·10	0·80
Green Gram	0·3	0·2	0·3	0·3	..
Black Gram	0·5	0·5	0·8	0·54	0·45
Bengal Gram	0·25	0·2	0·03	..	..
Coconut	0·11	..	..	..	..
Groundnut	..	..	..	0·15	..
Vegetables—					
Leafy	1·2	0·7	0·9	1·0	1·3
Non-leafy	2·6	2·2	1·7	1·3	1·7
Roots	0·7	0·2	0·35	0·2	0·5
Oil	0·3	0·23	0·18	0·15	0·1
Milk	9·0	8·0	3·5	3·2	3·3
Curds	2·6	1·4	2·1	2·0	0·5
Ghee	0·07	0·05	..	..	..
Mutton	..	0·7	..	0·32	0·65
Beef	..	..	..	..	..
Fish and Eggs	..	0·05	..	0·06	0·15
Sugar	2·0	1·8	1·0	1·0	0·5
Condiments	0·55	0·65	0·4	0·45	0·37

TABLE III—(*Continued*)

Article of Diets	Lower middle class (Muslim) non-vegetarian (6)	Poor class (Hindu) skilled labour non-vegetarian (7)	Poor class (Hindu) unskilled labour non-vegetarian (8)	(Hindu) conservancy workers non-vegetarian (9)	Poor class (Christian) unskilled labour non-vegetarian (10)	Poor class (Muslim) unskilled labour non-vegetarian (11)
Total cereals ..	16·05	18·8	16·2	20·93	16·3	14·7
Rice (Polished)	13·8	15·50	12·3	16·6	15·0	14·3
Wheat (flour) ..	2·25	..	..	0·13	..	..
Ragi	..	2·70	2·9	4·2	1·3	0·4
Cholam ..	..	0·60	1·0	..	..	..
Toial Pulses ..	1·06	0·78	0·51	1·33	0·93	0·3
Red Gram (Dhal Arhar ..	0·86	0·70	0·51	1·03	0·63	0·3
Green Gram ..	0·20	..	..	0·15	..	..
Black Gram ..	..	0·08	..	0·10	0·3	..
Bengal Gram ..	..	..	..	0·05	..	..
Coconut ..	..	..	..	..	..	..
Groundnut ..	..	..	..	..	..	..
Vegetables—						
Leafy ..	1·0	0·7	0·7	1·4	0·8	1·3
Non-leafy ..	2·4	1·1	0·7	1·6	1·0	1·5
Roots ..	..	..	0·2	0·2	1·0	0·8
Oil	0·03	0·03	0·05	0·1	0·05	0·05
Milk	2·5	..	..	0·3	..	..
Curds ..	0·7	0·93	0·80	1·2	1·3	..
Ghee ..	..	..	..	..	..	..
Mutton ..	..	0·14	0·05	..	0·41	..
Beef	0·9	..	..	1·05	..	0·35
Fish and Eggs..	0·5	..	0·02	..	0·10	..
Sugar ..	0·75	..	..	0·4	..	..
Condiments ..	0·50	0·25	0·15	0·3	0·15	0·30

TABLE IV

Mean Intake of Various Foodstuffs and Suggested Norms

(In oz. per consumption unit per day)

	Aykroid *et al.* (1940)	Wilson and Widdowson 1942 (Middle Class)	Wilson and Widdowson 1942 (Poor)	Ramalingaswami and Patwardan (1949)	Health Bulletin No. 23 (1951)
Rice ..	21·0	22·2	21·5	10·3	14
Wheat ..	..	1·4	0·2	0·92	
Cholam ..	..	..	..	0·74	
Ragi ..	..	..	..		
Others ..	..	..	..	1·78	
Pulses ..	1·4	1·4	1·0	1·6	3
Vegetables Leafy .	0·5	..	..	..	4
Non-leafy ..	1·0	5·3	3·6	2·6	3
Roots ..	..	3·8	2·2	2·6	3
Fruit ..	0·5	0·2	0·1	0·04	3
Vegetable Oil ..	0·15	0·27	0·2	0·4	2
Milk and Butter-milk ..	..	0·4	0·3	0·25	10
Butter ..	..	0·1	..	..	..
Meat ..	0·06	0·1	..	0·99	3
Fish .	..	0·3	0·5		
Egg ..	..	..	..	..	1
Sugar ..	..	0·1	0·1	1·4	2
Condiments ..	..	0·54	0·51	0·53	..
Coconut ..	..	..	..	0·5	..

The intake of proximate principles, minerals and vitamins were calculated from the Health Bulletin No. 23 (1951). The choline intake was calculated from the figures given by K. Ahmed *et al.* (1953) and Dakshinamurti (1955). The values as calculated are given in Table V.

Five samples (only 3 in case of Group 11) of the whole cooked food as it is eaten by one person were collected from each group. This was done on a voluntary basis. Only those families which were very co-operative were selected for this. The samples were homogenised in a waring blendor

TABLE

Chemical Composition of the

	Well-to-do (Hindu) vegetarian (1)	Well-to-do (Hindu) non-vegetarian (2)	Lower middle class (Hindu) vegetarian (3)	Lower middle class (Hindu) non-vegetarian (4)	Lower middle class (Christian) (non-vegetarian) (5)
Carbohydrate (gm.) ..	454 (127)	466 (118)	424 (108)	426 (108)	385 (121)
Protein (gm.)	61 (16)	57 (15)	52 (18)	52 (17)	50 (15)
Animal Protein (gm.) ..	8·8 (2·4)	11·8 (3·6)	3·7 (0·8)	5·4 (1·1)	7·6 (1·2)
Fat (gm.)	37 (13·2)	21 (9·8)	14 (4·0)	15 (4·1)	13 (3·6)
Calories	2263 (672)	2275 (594)	2032 (582)	2050 (620)	1838 (680)
Calcium (gm.)	0·711 (·23)	0·613 (·24)	0·410 (·20)	0·787 (·23)	0·59 (·26)
Phosphorus (gm.) ..	1·102 (·30)	1·074 (·32)	0·762 (·34)	0·955 (·29)	1·018 (·31)
Iron (mg.)	24 (7)	21 (6)	20 (6)	23 (7)	21 (5)
Vitamin A (I.U.) ..	1612 (320)	1121 (218)	1082 (261)	1097 (260)	1376 (259)
Thiamine (μ gm.) ..	773 (167)	686 (128)	518 (114)	901 (242)	685 (192)
Riboflavine (μ gm.) ..	898 (256)	855 (288)	536 (173)	510 (182)	520 (191)
Nicotinic acid (mg.) ..	6·3 (1·3)	7·1 (1·1)	7·1 (0·9)	6·8 (·9)	6·5 (1·3)
Choline (as choline chloride) (mg.)	444 (56)	491 (68)	375 (42)	392 (53)	448 (47)
Vitamin C (mg.)	52 (16)	34 (9)	44 (12)	49 (11)	64 (15)

Figures given in brackets

V

Diets of Different Classes of People

Lower middle class (Muslim) non-vegetarian	Poor class (Hindu) skilled labour non-vegetarian	Poor class (Hindu) unskilled labour non-vegetarian	(Hindu) conservancy workers non-vegetarian	Poor class (Christian) unskilled labour non-vegetarian	Poor class (Muslim) unskilled labour non-vegetarian
(6)	(7)	(8)	(9)	(10)	(11)
407	436	371	510	393	359
(105)	(96)	(75)	(125)	(110)	(120)
51	51	42	60	42	36
(16)	(18)	(14)	(18)	(13)	(14)
8·5	0·95	0·39	7·3	3·0	2·2
(2·5)	(·40)	(·18)	(2·8)	(1·4)	(1·0)
9	7·0	4·55	8·8	6·1	3·61
(2·2)	(1·8)	(1·8)	(2·7)	(2·1)	(1·7)
1900	1976	1653	2385	1793	1523
(521)	(563)	(427)	(589)	(496)	(710)
0·561	0·445	0·485	0·73	0·483	0·306
(·24)	(·20)	(·20)	(·28)	(·19)	(·12)
0·910	0·83	0·724	0·94	0·82	0·762
(·27)	(·35)	(·32)	(·31)	(·30)	(·30)
17	19	16	22	15	14
(4)	(4)	(5)	(6)	(4)	(4)
1053	678	575	1272	1022	1152
(243)	(181)	(173)	(623)	(223)	(198)
557	750	651	450	565	415
(132)	(133)	(122)	(198)	(118)	(103)
485	481	306	426	385	395
(172)	(168)	(141)	(173)	(142)	(152)
6·8	4·4	5·6	5·2	7·4	5·34
(1·1)	(0·8)	(0·7)	(1·2)	(1·1)	(1·1)
492	330	249	693	367	330
(62)	(41)	(36)	(78)	(55)	(45)
49	34	34	68	39	64
(12)	(10)	(11)	(13)	(8)	(12)

indicate standard deviation.

TABLE VI

Average Daily per capita *Consumption of Some Amino Acids in Grams*

Group	Protein	Trypto-phane	Leucine	Iso-leucine	Valine	Methio-nine	Lysine	Phenyl-alanine	Histidine	Arginine	Threo-nine	Cystine
1 Well-to-do Hindu Vegetarian	(60 gm.)	0·81	5·2	4·2	3·7	1·6	3·0	4·1	2·1	4·1	1·8	0·63
2 Well-to-do Hindu Non-vegetarian	(59 gm.)	0·74	5·2	3·8	4·3	1·4	3·1	3·4	2·0	3·9	1·9	0·64
3 Lower Middle Class Hindu Vegetarian	(52 gm.)	0·52	4·2	3·9	3·0	1·1	2·1	2·4	1·7	3·6	1·1	0·48
4 Lower Middle Class Hindu Non-vegetarian	(52 gm.)	0·61	4·2	3·0	3·0	1·1	2·2	2·3	1·9	3·1	1·6	0·47
5 Lower Middle Class Christian	(50 gm.)	0·63	4·2	2·7	2·9	1·0	2·2	2·2	1·5	2·8	1·7	0·5
6 Lower Middle Class Muslim	(51 gm.)	0·60	3·9	2·7	2·7	1·0	2·1	2·4	1·4	2·5	1·6	0·52
7 Poor Hindu Skilled Labour	(51 gm.)	0·55	3·8	2·6	2·7	1·1	2·0	2·0	0·8	2·4	1·6	0·50
8 Poor Hindu Unskilled Labour	(42 gm.)	0·50	3·2	2·1	2·2	0·8	1·6	1·7	0·8	2·3	0·9	0·40
9 Hindu Conservancy Worker	(60 gm.)	0·81	5·1	4·5	3·7	1·3	2·8	2·1	1·0	3·3	1·8	0·59
10 Poor Christian Unskilled Labour	(42 gm.)	0·60	3·9	2·7	2·7	0·9	2·1	2·3	1·3	2·5	1·5	0·46
11 Poor Muslim Unskilled Labour	(36 gm.)	0·50	3·2	2·2	2·2	0·9	1·8	1·8	0·8	2·4	1·4	0·40
12 Recommended intake (Rose)	..	0·50	2·2	1·4	1·6	2·2	1·6	2·2	..	..	1·0	..
13 American Urban Families Annual Income $2500 - 2999	(94 gm.)	1·2	8·2	5·7	5·6	4·0*	5·8	5·1	2·3	5·6	3·9	..
14 American Urban Families Annual Income $0 - 499	(74 gm.)	0·9	6·5	4·2	4·2	3·0*	4·0	4·1	1·7	4·2	2·8	..
15 Total Population (1937—41)	(89 gm.)	1·1	12·6	3·7	3·9	4·1*	5·2	4·7	2·0	4·7	3·6	..

* Values for Methionine+Cystine.

and preserved in a refrigerator till used. Each sample was analysed for its amino acid contents after acid and alkaline hydrolyses. The following amino acids were estimated: tryptophane, leucine, isoleucine, arginine, valine, methionine, lysine, phenylalanine, histidine, threonine and cystine. The chromatographic method of Giri and Rao (1952) as modified by Dakshinamurti (1954) was used for the estimation of amino acids. Methionine was estimated by the method of Mc Carthy and Sullivan (1941) and cystine by that of Block and Bolling (1951). The averages for each group along with the intake recommended by Rose (1949) and the average *per capita* consumption of essential amino acids in the United States of America are given in Table VI.

Results and Discussion

The main cereal consumed is polished rice though wheat, ragi and cholam are eaten by different sections in much smaller amounts. One striking feature is the reduction in the total cereals intake recorded in post-war diet surveys. A comparison of the values given in Tables III and IV poses the question whether the continued scarcity of essentials and their consequent rationing during the war coupled with the almost famine conditions of the post-war years due to the continued failure of monsoon have left a permanent effect on the quantity of food eaten by people.

The caloric intake in groups 8, 10 and 11 is really puzzling. The possibility of obtaining unaccounted extraneous sources of nourishment is greatly limited because of the low income of the groups.

The protein intake is generally low but the values for the classes of unskilled labour in all the communities are alarmingly low. The same is the case with the animal protein intake.

Fat consumption varies with income. With a rise in income there is a rise in the intake of fat. But fat consumption in all the groups except 1 and 2 is extremely low.

Calcium intake in all the groups is much lower than the recommended level. The actual intake of vitamins must be much lower than those appearing in Table V because of the methods of cooking. There is no definite recommendation in regard to the choline necessary. The choline intake varies from 249 mg. to 502 mg. It is greater in nonvegetarian diets.

Rose (1949) has demonstrated that the adult man requires eight amino acids in the diet to meet the demands for the replacement of tissue catabolism. These recommended values are based on the assumption that the diet furnishes sufficient nitrogen for the synthesis of the non-essential amino acids.

Table VI indicates the extremely low level of amino acid intake as compared with that of even the poorest sections in the U.S.A. Even as compared with the modest recommendations of Rose, the diets of group 8 show marginal and sub-marginal levels of tryptophane, methionine, lysine, phenylalanine and threonine and group 11 of tryptophane, methionine, lysine and phenyl alanine. The methionine recommendation of Rose cannot be taken as specific because the choline content of the diet has not been taken into consideration by him and choline has a sparing action on methionine. Nutritional errors such as low caloric intake, deficiency in dietary protein, starvation, lack of certain vitamins or almost any illness will increase loss of tissue nitrogen through excessive catabolism (Keys *et al.*, 1950 and Lynch, 1951). Excessive tissue protein catabolism represents loss and marked imbalance in the enzyme systems (Miller, 1948). Singhal *et al.* (1953) have shown that massive fatty infiltration of the liver occurs under conditions of threonine and lysine deficiency. These are two of the limiting amino acids in the diet of groups 8 and 11. These facts raise the question: Could it be that the amino acid imbalance, more particularly the deficiency of lysine, threonine and methionine is the cause for the continuing liver damage of some sort? The present investigation is an attempt in formulating an experimental regimen simulating the diets of group 8 and 11 with a view to assessing the possibility of producing liver injury in the albino rat. It is hoped that this further work will throw light on the high incidence of the diseases of the liver in this area.

SUMMARY

A diet survey has been carried out in Vellore town. The survey included 507 families divided into 11 groups.

The caloric intake for the poorest section is appalingly low. Protein and fat intakes are also extremely low. The diet of the poor classes is generally deficient in quality as well as quantity. It is not only a question of lack of vitamins but also of fats, proteins, minerals and even caloris.

The amino acid pattern of the diets of poor people is discussed in relation to its possible role in producing liver injury.

REFERENCES

Ahmed, K., Karim, M. A. and De, H. N. — *Ind. J. Med. Res.*, 1953, **41** (4), 441.

Aykroid, W. R., Krishnan, B. G. — *Ibid.*, 1936, **24**, 667.

———, ———, Passmore and Soundararajan, A. R. — *Ind. Med. Res. Memoir No.* 32, 1940.

Block, R. J. and Bolling .. — *The Amino Acid Composition of Proteins and Food*, 1951, Clarke C. Thomas, Springfield, Ill., U.S.A.

Brock, J. F. ..	*Annals of the N.Y. Acad. Sci.*, 1954, **56** (6), 696.
Canadian Studies on Nutrition	*Canadian Pub. Health Jour.*, 1941, **32**, 37.
Dakshinamurti, K. ..	*Curr. Sci.*, 1954, **23**, 89.
———— ..	*Ibid.*, 1955, **24**, 194.
Davies, J. N. P. ..	*Ann. Rev. Med.*, 1952, **3**, 99.
Earnest, R. Jaffe, Eleanor M. Humphreys, Earl P. Benditt and Robert R. Wissler	*Arch. Path.*, 1949, **47**, 411 (consulted but not quoted in text).
Fernando, P. B., Mc. Donza, O. R. and Rajasurya, P. K.	*Lancet*, 1948, **255**, 205.
Gilbert, G. and Gilman, J. ..	*Science*, 1944, **99**, 398.
Gilman, J. and Gilman, T. ..	*Perspectives in Human Malnutrition*, 1951, Grune and Stratton, New York (consulted but not quoted in text).
Giri, K. V. and Rao, N. A. N...	*J. Ind. Inst. Sci.*, 1952, **34**, 95.
Hance, J. B. ..	*Guy's Hosp. Rep.*, 1928, **78**, 379.
Health Bulletin No. 23 ..	*The Nutritive Value of Indian Foods and the Planning of Satisfactory Diets*, 1951, Government of India Press, Simla.
Huges, J. A. ..	*Ind. J. Med. Res.*, 1933, **21**, 353.
Keys, A. Brozek, J. Henschel, A., Mickelson, O and Taylor, H. L.	*Biology of Human Starvation*, Vol. I, 1950, Univ. of Minnesota Press, Minneopolis.
League of Nations ..	*Quart. Bull. Health Org.*, 1932, **1**, 476.
Lynch, H. D. and Snively, W. D.	*J. Am. Med. Assoc.*, 1951, **147**, 115.
Mc Carthy and Sullivan ..	*Jour. Biol. Chem.*, 1941, **141**, 871.
Mc. Farlane and Brandy, W. J...	*Brit. M. J.*, 1945, **1**, 838.
Menon, T. B. and Annamalai, D. R.	*Ind. J. Med. Res.*, 1935, **22**, 827.
Miller, L. L. ..	*Journ. Biol. Chem.*, 1948, **172**, 113.
Platt, B. S. ..	*Nutrition in British West Indies*, Great Britain Colonial No. 195 Lond. H.M.'s Stationary Office, 1945.
Ramalingaswami, V. and Patwardan, V. N.	*Ind. J. Med. Res.*, 1949, **37**, 51.
Rose, W. C. ..	*Fed. Proc.*, 1949, **8**, 546.
Singhal, S. A., Hazan, S. J. Syndenstricker, V. P. and Julia M. Little John	*Jour. Biol. Chem.*, 1953, **200**, 867.
Tirumurti and Rao, M. V. R. ..	*Ind. Med. Gaz.*, 1934, **69**, 74.
Vijayaraghavan, P. K. and Patwardan, V. N.	*Ind. J. Med. Sci.*, 1952, **6**, 696.
Wahi, P. N. ..	*Arch. Path.*, 1949, **47**, 119.
Willson, D. C. and Widdowson, E. M.	*Ind. Med. Res. Memoirs, No.* 34, 1942.
Witts, L. J. ..	*Brit. M. J.*, 1947, **1**, 1.

THE ENZYMES OF SOME ELASMOBRANCHS FROM BOMBAY

III. AMYLASES of *Scoliodon sorrakowah* and *Sphyrna blochii*.

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A vast amount of information on the amylases of tissues of higher animals and plants and those of yeasts molds and bacteria is available. The amylases of fish have not been studied much until recent times, except for the work of Yung (1898), Sullivan (1907), Kenyon (1925) and Yonge (1931), showing the presence of amylopsin in fish pancreas. The present investigation deals with the amylases of *S. sorrakowah* and *S. blochii*, the two varieties of Sharks from Bombay Waters.

Methods and Material

(i) 6% Aqueous extracts of various tissues were prepared by grinding the tissue in a mortar with glass powder. The extracts autolysed at 15° C. for 18 hours with toluene as the preservative were squeezed through muslin, and the filtrate in each case used as the source of enzyme.

pH 1 to 3·0 and 9 to 10·0 was maintained by M/10 glycine buffer, that from 4 to 5·0 by M/5 acetate buffer and from 6 to 8·0 by M/10 phosphate buffer.

The reaction mixture "in the proportion $2n$ c.c. Sub-strate, $2n$ c.c. buffer and n c.c. enzyme preparation (boiled in the case of control)" was set up at 37° C.

(ii) *Reagents.*—(*a*) Shaffer Somogyi copper reagent: 25 gm. anhydrous Na_2CO_3 and 25 gm. Rochelle salt were dissolved in 500 c.c. of water without the application of heat. To this 100 c.c. of 12% $CuSO_4$ $5H_2O$ (A.R.) solution were added by a pipette, its tip being kept under the solution of carbonate and tartrate to avoid losing of CO_2·20 gm. $NaHCO_3$, 160 gm. Na_2SO_4, 12 gm. KI and KIO_3 solution in volume according to the quantity of sugar to be analysed (here 120 c.c. of 0·4 N·KIO_3 were used) were added. Reagent was diluted to a litre, mixed and filtered, a few drops of toluene added as the preservative and stored in a brown bottle away from dust.

(*b*) Standard maltose solution was prepared by dissolving anhydrous maltose (Merck) in 0·25% benzoic acid solution.

(*c*) Stock iodine solution was prepared by dissolving 5·5 gm. of I_2 and 11·0 gm. of KI in 250 c.c. of water.

(*d*) *Dilute iodine solution.*—15 c.c. of stock iodine solution and 8 gm. of KI were diluted to 200 c.c. with water so that 1 c.c. sol. = 1·65 mg. I_2.

(*e*) *Standard dextrin solution.*—0·6 gm. Merck's reagent dextrin was suspended in small amount of cold water and then transferred to 400 c.c. of boiling water, after cooling the solution was made to a litre. The solution preserved under toluene in refrigerator keeps for several weeks. Red brown colour produced when a c.c. of dextrin solution is mixed with 5 c.c. of dilute iodine solution serves for colour comparisons.

(*f*) One gm. of corn amylum (Maize starch) made into a thin paste with little cold water was added slowly with constant stirring to hot water and the solution was boiled for fifteen minutes and cooled. 1% starch sol. adjusted to requisite pHs. served as the substrate. It was prepared fresh when required.

(iii) *Determination of enzyme activity.*—The saccharogenic activity of enzyme was determined by the estimation of maltose produced according to Shaffer and Somogyi (1933 and 1937). To draw a standard reference curve for maltose 2 c.c. sample containing 0·25 to 9·0 mgm. maltose were pipetted in a pyrex test-tube containing 5 c.c. of copper reagent. The contents were mixed and kept for 15 minutes in a boiling water-bath, then the tube was immersed in cold water and finally in freezing mixture to bring the contents to 1 to 4° C. One c.c. of $5N \cdot H_2SO_4$ was then introduced in test-tube, mixed and iodine liberated was titrated against $N/100\ Na_2S_2O_3 \cdot 5H_2O$ with starch as an indicator. 4 c.c. reaction mixture were pipetted at zero time (*i.e.*, at the moment the enzyme was introduced) and after 20 minutes and were added to 5 c.c. of copper reagent in separate test-tubes, and other treatments followed subsequently as described. Activity of enzyme is expressed as the mgm. maltose produced by the enzyme present in 100 mgm. dry tissue, under the specified conditions.

The liquefying activity of amylases was measured viscometrically, the change in the initial rate of flow (in seconds) of 100 mgm. starch caused by the enzyme present in 30 mgm. dry tissue in 20 minutes at 37° C. being an index of enzyme activity.

The dextrinogenic activity of enzyme was followed by Sandstedt Kneen and Blish's (1939) modified Wohlgemuth procedure. One c.c. of reaction mixture removed at definite time interval was mixed with 5 c.c. of dilute iodine solution and colour comparisons were made with red brown colour

of standard dextrin solution, for the time interval " t " necessary for the end of dextrinization caused by the amount of enzyme " d.wt." represented by the dry weight of the enzyme preparation, amylase units were calculated as

$$\text{Amylase units} = \frac{\text{Wt. of starch} \times 60}{\text{t} \times \text{d.wt.}}$$

RESULTS

General survey of amylolytic activity of various tissues of the two fishes was made by the study of the dextrinogenic activity. Reactions were run at pH 7·0 and the readings were taken after 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 125, 140, 160, 180, 240, 280, 360 and 1,440 minutes to note the end of dextrinization. The reaction time when the red brown and achromic stage was reached for different amylase preparations is given in Table I.

TABLE I

General Survey of Amylolytic Activity by Modified Wohlgemuth's Procedure

Reaction mixture "containing 10 c.c. substrate + 10 c.c. Buffer (pH 7 0)+ 5 c.c. enzyme preparation" was set up at 37° C.

Fish	Organ	(Figures in bracket indicate amylase units) Time in minutes for	
		Red brown stage	Achromic stage
Scoliodon sorrakowah ..	Viscera	15 (13·33)	30
	Liver	40 (5·00)	60
	Spleen	55 (3·64)	240
	Kidney	55 (3·64)	240
	Heart	160 (1·25)	280
	Brain	160 (1·25)	280
	Muscle	..	1440
	Ampullæ of lorenzini	..	..
Sphyrna blochii ..	Viscera	15 (13·33)	25
	Liver	55 (3·64)	100
	Spleen	90 (2·22)	180
	Kidney	90 (2·22)	180
	Heart	140 (1 43)	240
	Brain	140 (1·43)	280
	Muscle	..	1440
	Ampullæ of lorenzini	..	..

TABLE II A

Saccharogenic Activity of Amylases at Various pHs.

Reaction mixture "containing 6 c.c. substrate + 6 c.c. buffer of required pH + 3 c.c. enzyme preparation" was kept at 37° C. for 20 minutes.

Animal	pH	Enzyme activity/100 mgm. dry tissue, in terms of mgm. maltose							
		Organ							
		Pancreas	Liver	Spleen	Kidney	Brain	Muscle	Stomach	Intestine
Fish *Scoliodon sorrakowah*	2·0	..	1·16	..	0·0	..	..	0·0	..
	3·0	3·52	5·79	2·32	4·63	0·0	0·0	0·0	0·0
	4·0	8·15	15·05	5·29	8·15	0·0	0·0	0·0	4·63
	5·0	79·64	27·77	8·15	18·52	6·94	0·0	0·0	12·74
	6·0	243·20	16·20	17·36	9·26	0·0	0·0	0·0	0·0
	7·0	185·20	48·61	28·94	2·32	5·79	5·79	0·0	4·63
	8·0	11·10	33·57	4·63	2·32	0·0	0·0	0·0	0·0
Fish *Sphyrna blochii*	2·0	..	0·0	..	..	0·0	..	0·0	..
	3·0	6·94	4·63	3·52	4·63	0·0	0·0	0·0	0·0
	4·0	25·46	11·57	5·79	6·94	0·0	0·0	0·0	0·0
	5·0	127·40	34·71	8·15	22·01	6·94	0·0	0·0	0·0
	6·0	87·96	15·05	18·52	8·15	0·0	0·0	0·0	0·0
	7·0	182·80	37·03	32·41	2·32	2·32	4·63	0·0	6·94
	8·0	11·10	23·15	4·63	0·0	0·0	1·16	0·0	1·16
	9·0	74·08	15·05	0·0	0·0	0·0	0·0	0·0	0·0
Pig	2·0	..							
	3·0	5·79							
	4·0	50·93							
	5·0	189·80							
	6·0	402·80							
	7·0	240·70							
	8·0	125·00							
	9·0	74·08							

To know the distribution of amylases in different tissues of the two fishes and pig pancreas activity pH curve for each was studied. The saccharogenic and liquefying activities were determined and the results are represented by Tables II A and II B.

TABLE II B

Liquefying Activity of Amylases, at Various pHs.

Reaction mixture "containing 10 c.c. substrate + 10 c.c. buffer of required pH + 5 c.c. enzyme preparation" was set up at 37° C. for 20 minutes.

Animal	pH	Enzyme activity/30 mgm. dry tissue in terms of seconds			
		Organ			
		Pancreas	Liver	Spleen	Kidney
Fish	2·0	..	0·8	..	0·0
Scoliodon sorrakowah	3·0	4·0	7·0	0·0	1·0
	4·0	15·0	10·0	0·0	7·0
	5·0	26·0	23·0	2·0	12·0
	6·0	81·0	9·0	5·0	4·0
	7·0	64·0	12·5	9·0	0·5
	8·0	42·2	3·0	1·0	0·0
	9·0	30·3	1·0	0·0	..
Fish	2·0	..	0·5	..	0·0
Sphyrna blochii	3·0	5·0	7·5	0·0	0·0
	4·0	15·0	9·5	0·0	7·5
	5·0	68·5	26·0	1·0	14·0
	6·0	39·0	10·0	4·0	6·0
	7·0	52·0	12·5	8·0	0·0
	8·0	30·0	5·5	1·3	0·0
	9·0	19·0	2·0	0·0	..
Pig	3·0	11·0			
	4·0	21·0			
	5·0	53·0			
	6·0	94·0			
	7·0	64·0			
	8·0	40·0			
	9·0	29·0			

The distribution of α- and β-amylases in pancreas, liver, spleen and kidney of the two fishes and pig pancreas has been worked out by the application of Ohlsson's (1922, 1926 and 1930) technique. According to this technique original extract represented a mixture of α- and β-amylases, the original extract adjusted to pH 6·5 to 6·6 and then heated to 70° C. for 15 minutes is the source of α-amylase and the original extract brought to 1 to 2° C. and then adjusted to pH 3·4 represented β-amylase. Amount of maltose produced being the measure of enzyme activity, the results are embodied in Table III.

Effect of the presence of Ca^{++} in varying concentration in enzyme extract, on the application of Ohlsson's technique to pancreatic amylases of pig and *S. sorrakowah* has been studied. Enzyme activity was followed reductometrically by carrying out the reactions at pH 6·0, at 37° C. for 20 minutes. The results are shown by Figs. 1 A and and 1 B.

To study the solubility of amylases of pancreas, liver, spleen and kidney of *S. sorrakowah* and pancreas of *S. blochii*, homogenised tissue in each case was extracted separately with water and 10% (V/V) aqueous glycerol to prepare 6% extracts. All extracts were autolysed overnight at 15° C., squeezed through muslin and then filtered under suction. Residue in each case was suspended in the amount of extractant equal to that of the extract from which residue was obtained. Cloth filtrates, buchner filtrates and residue suspensions were tested for enzyme activity reductometrically, in a usual way by carrying out the reactions at the respective activity pH optimum or optima as exhibited by amylases of different organs. The results are given in Table IV.

Experiment was carried out to study the effects of varying H^+ concentration of pancreatic desmo- and lyo-amylases of *S. sorrakowah* and *S. blochii*. The solution of amylase under investigation was brought to 1 to 2° C. and was maintained at different pHs. for 15 minutes. The various test solutions thus prepared were used as the source of enzyme. Amylase activity was estimated reductometrically. The reactions in each case were run at the respective activi y pH optimum exhibited by the enzyme. The results are given in Table V. Percentage destruction of amylase by varying pH of enzyme solution is represented by Fig. 2.

TABLE III

Activity of α- and β-amylases, α-amylase and β-amylase at Various pHs.

Reaction mixture "containing 10 c.c. substrate + 10 c.c. buffer of required pH + 5 c.c. enzyme preparation" was set up at 37° C. for 20 minutes.

Animal	Organ	Amylase type	Enzyme activity/100 mgm. dry tissue in terms of mgm. maltose					
			pH					
			3·0	4·0	5·0	6·0	7·0	8·0
Fish : *Scoliodon sorrakowah*	Pancreas	α- & β-	2·29	5·83	57·90	180·60	113·30	46·25
		α-	0·0	0·00	23·12	64·79	30·00	11·66
		β-	0·0	0·0	43·95	120·40	60·00	20·83
	Liver	α- & β-	0·0	13·96	25·44	13·96	39·38	20·83
		α-	0·0	3·54	17·92	6·54	2·29	0·0
		β-	0·0	0·0	0·0	4·58	25·44	5·83
	Spleen	α- & β-	0·0	6·87	9·17	16·25	25·42	4·58
		α-	0·0	0·0	0·0	0·0	0·0	0·0
		β-	0·0	0·0	2·29	4·58	11·66	3·54
	Kidney	α- & β-	0·0	6·87	18·54	6·87	2·29	0·0
		α-	0·0	0·0	6·87	2·29	2·29	0·0
		β-	0·0	0·0	0·0	0·0	0·0	0·0

		β-	0·0	0·0	0·0	0·0		
Fish: *Sphyrna blochii*	Pancreas	α- & β-	11·66	43·95	127·3	71·66	159·8	92·28
		α-	0·0	4·58	78·24	27·71	0·0	0·0
		β-	0·0	0·0	23·12	55·62	141·2	74·15
	Liver	α- & β-	0·0	13·96	27·71	20·83	43·95	20·83
		α-	0·0	4·58	16·25	2·29	0·0	0·0
		β-	0·0	0·0	0·0	11·66	39·59	9·17
	Spleen	α- & β-	0·0	2·29	9·17	16·25	27·71	11·66
		α-	0·0	0·0	0·0	0·0	0·0	0·0
		β-	0·0	0·0	0·0	9·17	18·54	4·78
	Kidney	α- & β-	0·0	6·87	18·54	9·17	0·0	0·0
		α-	0·0	4·58	18·54	6·87	0·0	0·0
		β-	0·0	0·0	0·0	0·0	0·0	0·0
Pig	Pancreas	α- & β-	34·78	60·20	94·99	259·10	210·60	145·80
		α-	0·0	13·96	27·71	78·74	32·44	18·54
		β-	0·0	20·83	43·95	166·60	69·36	23·12

TABLE IV

Water and 10% *Aqueous Glycerol as Extractants of Amylases*

Reaction mixture "containing 6 c.c. substrate + 6 c.c. buffer of required pH + 3 c.c. enzyme preparation" was kept at 37° C. for 20 minutes.

Fish	Organ	pH	Enzyme activity/100 mgm. dry tissue in terms of mgm. maltose					
			Water extract			Glycerol extract		
			Cloth filtrate	Buchner filtrate	Residue sus	Cloth filtrate	Buchner filtrate	Residue sus
S. blochii	Pancreas	5·0	180·6	53·32	127·3	180·6	57·9	129·6
		7·0	208·3	208·3	25·42	208·3	201·3	23·12
S. sorrakowah	Pancreas	6·0	194·4	152·7	48·54	199·2	157·5	48·54
	Liver	5·0	27·71	6·87	25·42	30·0	6·87	27·71
		7·0	41·66	39·38	4·58	46·25	43·95	4·58
	Kidney	5·0	20·83	2·29	20·83			
	Spleen	7·0	27·71	30·0	0·0			

TABLE V

Effects of Varying pH of lyo- and desmo-form Pancreatic Enzyme Solutions on lyo- and desmo-amylases

Reaction mixture "containing 6 c.c. substrate + 6 c.c. buffer of required pH + 3 c.c. enzyme solution" was set up at 37° C. for 20 minutes.

Enzyme activity/100 mgm. dry tissue in terms of mgm. maltose*

pH of amylase solution for 15 minutes	*Scolidon sorrakowah*		*Sphyrna blochii*	
	Lyo-amylase	Desmo-amylase	Lyo-amylase	Desmo-amylase
	Reaction pH			
	6·0	6·0	7·0	5·0
2·5	22·29 (13·9)	..	45·8 (25·0)	..
3·1	48·54 (29·5)	..	69·36 (37·9)	..
3·4	196·6 (119·6)	9·17 (18·8)	199·20 (108·9)	11·66 (9·0)
4·0	155·00 (94·3)	23·12 (47·6)	175·80 (96·1)	27·71 (21·4)
4·5	120·40 (72·2)	25·42 (52·4)	150·40 (82·2)	46·25 (35·7)
5·0	157·50 (95·8)	41·66 (85·8)	175·80 (96·1)	113·30 (87·5)
6·0	164·40 (100·0)	48·54 (100·0)	182·90 (100·0)	129·10 (100·0)
7·0	139·00 (84·5)	41·66 (85·8)	143·50 (78·5)	111·00 (85·7)
8·0	120·40 (73·4)	39·4 (81·1)	118·10 (64·6)	94·99 (73·3)
9·0	118·10 (71·9)	..	78·74 (43·0)	87·90 (67·8)

* ( ) % amylase in lyo- or desmo-form enzyme solution.

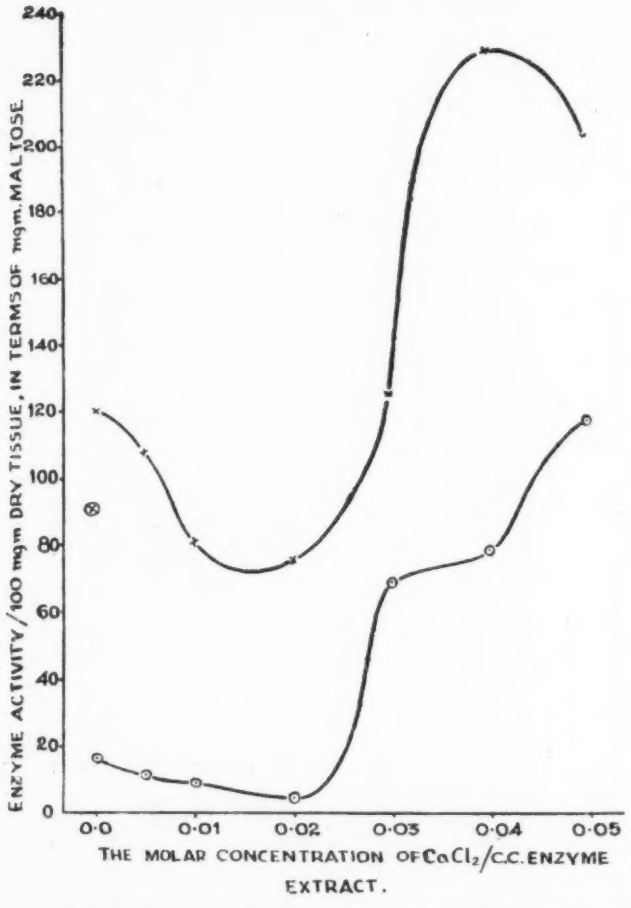

FIG. 1 A. Effect of Ca^{++} in varying concentration on the application of Ohlsson's technique to pancreatic amylases of pig.

⊗ α- and β-amylases
⊙–⊙–⊙ α-amylase
x-x-x- β-amylase

DISCUSSION

A general survey (Table I) of amylolytic activity of the various tissues, of the two fishes shows that the activity is maximum in pancreas, minimum in intestine, muscle, heart and brain, intermediate in liver, spleen and kidney and absent in stomach and ampullæ of lorenzini.

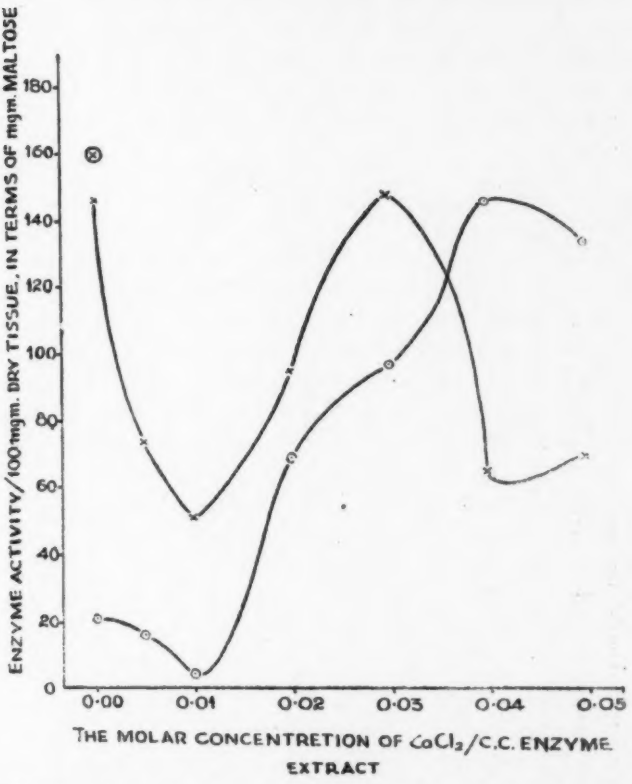

FIG. 1 B. Effect of Ca^{++} in varying concentration on the application of Ohlsson's technique to pancreatic amylases of *S. sorrakowah*.

⊗ α- and β-amylases
O-O-O α-amylase
x-x-x β-amylase.

It will be seen from the results in Table II A and II B that saccharogenic and liquefying activities of amylases of *S. blochii* pancreas and liver of both the fishes exhibit maxima at pH 5·0 and 7·0, of *S. sorrakowah* and pig pancreas exert optimum at pH 6·0 and in both the fishes those of kidney and spleen act best at pH 5·0 and 7·0 respectively. Optimal saccharogenic activity of intestinal and brain enzymes is at pH 5·0 and 7·0 and of muscle is at pH 7·0.

The two activity-pH-optima suggest the probable presence of two types of amylases. The higher saccharification and lower liquefaction at pH 7·0

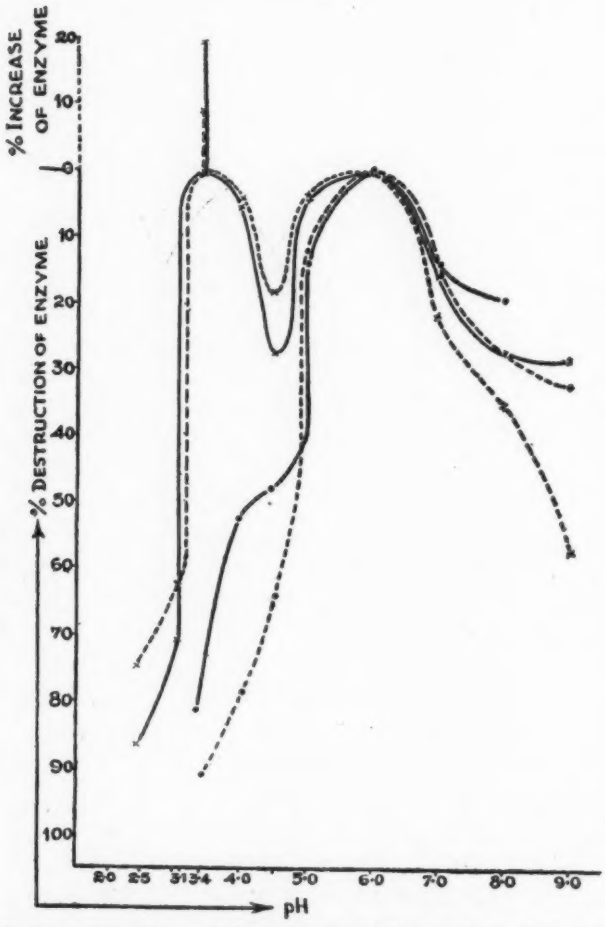

FIG. 2. % destruction of lyo- and desmo-form pencreatic amylases of fish, caused by varying H^+ concentration of enzyme solutions at 1 to 2° C.

S. sorrakowah	x—x—x	Lyo-amylase
	-●-●-●-	Desmo-amylase
S. blochii	-x-x-x-	Lyo-amylase
	-●-●-●-	Desmo-amylase

than at pH 5·0 give an indication of β-amylase to be acting best at pH 7·0 and α- at 5·0 or there may be a mixture of α- and β-amylases represented by two isodynamic systems at pH 5·0 and 7·0 respectively. The only one activity pH optimum tends to show the presence of either α- or β-amylase or there may be a mixture of the two acting best at the same pH.

Table III shows that *S. blochii* pancreatic and *S. blochii* and *S. sorrakowah* liver α- and β-amylases show optimum activity at pH 5·0 and 7·0 respectively, whereas those of *S. sorrakowah* and pig pancreas show maximum activity at pH 6·0. Kidney amylase is an α-amylase acting best at pH 5·0 and that of spleen is a β-amylase with optimal action at pH 7·0.

As per application of Ohlsson's technique (Fig. 1 A), pig pancreatic extract retains much of α-amylase, and β-amylase gets destroyed, when it is adjusted to pH 6·5 to 6·6 and heated to 70° C. for 15 minutes, while the extract at 1° to 2° C., if adjusted to pH 3·4, β-amylase is unaffected α-being destroyed. But when Ca^{++} is introduced in terms of increasing doses of $CaCl_2$ in enzyme extract, thermostability of α-amylase and acid stability of β-amylase starts decreasing, maximum decrease in each case is effected when Ca^{++}/c.c. extract is 0·02 M. Further addition of Ca^{++}, now causes increase in enzyme activity. Maximum increase in case of α-occurs when Ca^{++}/c.c. extract is 0·05 M. and that in the case of β-occurs when it is 0·04 M/c.c. extract.

A similar pattern of results (Fig. 1 B) has been observed with *S. sorrakowah* pancreatic amylases. Here the thermostability of α- and acid stability of β- fall to the maxima when Ca^{++}/c.c. extract is 0·01 M. Further addition of Ca^{++} turns out in the rise of enzyme activity. The optimum increase of α-amylase is indicated at 0·4 M. Ca^{++}/c.c. extract, and that of β-amylase at 0·03 M. Ca^{++}/c.c. extract.

The above results suggest the exchange of thermo-stability and acid-lability of α-amylase with the thermolability and acid stability of β-amylase, *i.e.*, the probable interconversion α-amylase ⇋ β-amylase under the influence of Ca^{++} as indicated in the case of wheat amylases (1943).

The study of the solubility (Table IV) of amylases of pancreas liver spleen and kidney of *S. sorrakowah* and pancreas of *S. blochii* shows that both water and 10% aqueous glycerol are equivalent extractants of enzymes. α-type is a desmo-amylase and β-type a lyo-amylase.

The results in Table V and Fig. 2, show that in both the fishes destruction of desmo- and lyo-amylases starts when H^+ concentration of enzyme preparations is taken below or raised above pH 6·0 (original pH of enzyme extract). The destruction of desmo-amylase at pH 3·4 is 90·90%. The loss of lyo-amylase however, proceeds upto pH 4·5, at pH 4·0 it is less than what it was at pH 4·5, at pH 3·4 there is no fail in enzyme activity but the amount of active amylase content in solution is increased by 19·6% in *S. sorrakowah* and by 8·9% in *S. blochii*. The slight shift from pH 3·4 towards acidity is now followed by great losses in enzyme activity.

The acid lability of desmo-amylase and acid stability of lyo-amylase show that the former is predominantly of α-type and the latter of β-type.

The increase in active amylase content of pancreatic lyo-amylase solutions, when they are brought to 1 to 2° C. and adjusted to pH 3·4 may be due to the probable presence of Zymogen precursor of enzyme.

SUMMARY

1. In *S. sorrakowah* and *S. blochii* amylolytic activity is maximum in pancreas, minimum in intestine, muscle, heart and brain, intermediate in liver, spleen and kidney and absent in stomach and ampullæ of lorenzini.

2. Pancreas and liver of both the fishes and pig pancreas contain α- and β-amylases. Kidney amylase is an α-amylase and spleen amylase is a β-amylase.

3. In *S. sorrakowah* and pig there seems to be probable interconversion of pancreatic α-amylase ⇋ β-amylase under the influence of Ca^{++}.

4. Both water and aqueous glycerol are equivalent extractants of enzyme. Desmo-amylase seems to be predominantly of α-type and lyo-amylase of β-type.

5. Pancreatic lyo-amylase of both the fishes shows the presence of pro-amylase.

REFERENCES

1. Kenyon, W. A. .. *Bull. Bur. Fisheries*, 1925, **41**, 181–200.
2. Kneen, E., Sandestedt, R. M. and Hollenbeck, C. M. *Enzymes and Their Role in Wheat Technology*, Interscience, N.Y., 1943, **20**, 399.
3. Ohlsson, E. .. *Compt. Rend. Soc. Biol.*, 1922, **87**, 1183.
4. ———— .. *Compt. Rend. Trav. Lab. Carlsberg Ser. Chim.*, 1926, **16**, 7.
5. ———— .. *Z. Physiol. Chem.*, 1930, **189**, 17.
6. Sullivan, M. X. .. *Bull. U.S. Bur. Fisheries*, 1907, **28**, 3–27.
7. Shaffer, P. A. and Somogyi, M. *J. Biol. Chem.*, 1933, **100**, 695.
8. Somogyi, M. .. *J. Biol. Chem.*, 1937, **117**, 771.
9. Sandstedt, R. M., Kneen, E. and Blish, M. J. *Cereal Chem.*, 1939, **16**, 712.
10. Yung, E. .. "*Actes Soc. Helvet. Sci. Nat. Geneve Compt. Rend. Des. Seances de 1*," *Academie des Sciences Paris*, 1898, **127**, 77.

DEVELOPMENTAL STUDIES OF *PHYLLANTHUS NIRURI* LINN. AND *P. RETICULATUS* POIR. (EUPHORBIACEÆ) WITH SPECIAL REFERENCE TO THE ORIGIN AND NATURE OF AXILLARY VEGETATIVE BUDS

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Introduction

Prain (1903) has listed ten Bengal species of *Phyllanthus* Linn. of which the following six are common in East Bengal: *P. niruri* Linn., *P. reticulatus* Poir., *P. Emblica* Linn., *P. urinaria* Linn., *P. simplex* Retz. and *P. distichous* Muell.

The primary branches of *P. niruri* often *resemble* pinnately compound leaves. But in the axil of each pinna (simple leaf) one (*P. niruri*), or a cluster (*P. reticulatus*) of flower-buds are borne. Moreover, a pair of scaly stipules are present at the base of the short petiole of these leaves. Most often in the axil of these primary branches, which are of limited growth, more than one vegetative buds (secondary and tertiary branches) arise slightly extra-axillary in position. The presence of these branches often misguides a casual observer in believing the primary branches as true compound leaves (Fig. 1).

The plant *P. niruri* is very often used in class rooms to demonstrate the differences between a compound leaf and a branch with simple leaves on account of flower buds or fruits borne in the axils of these leaves.

The present studies were undertaken especially to re-examine the morphological nature of these leaf-like branches on anatomical evidence.

Materials and Methods

The following two species were studied anatomically: *P. niruri* and *P. reticulatus*. Materials of *P. niruri* were collected from Dacca University Botanical Garden and F.H.M. Hall compound, and those of *P. reticulatus* from different hedges in and around Dacca City.

For developmental studies, particularly of the axillary branches, the growing apex, nodes, branch-nodes and branch tips were killed and fixed

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in FAA solution, dehydrated and embedded in paraffin according to schedule. Microtome sections of these materials were cut at 6 to 15 μ with a rotary microtome. The sections, both t.s. and l.s., were stained with safranin and fast green combination and made permanent in canada balsam.

Freehand sections of the nodes of the main axis and branches were also cut to study the vascular supply to the leaves and buds. The sections were suitably stained, dehydrated and mounted in canada balsam. All figures have been drawn with a camera lucida.

OBSERVATIONS

Morphology

P. niruri Linn.—(Fig. 1)—An annual weed, 6–8 inches high with spirally arranged branches which are naked and smooth below. The branches arise in the axils of three-fid scaly structures, the leaves (Fig. 1 *c*). More than one branch buds are often seen to arise in the axils of these branches (Fig. 1 *a*). The simple leaves on the primary branches are arranged distichously on each branch which is of limited growth in *P. niruri*. Axillary buds of these leaves develop into single flowers but never into vegetative branches (Fig. 1 *b*).

P. reticulatus Poir.—(Fig. 2)—A large much branched diffuse subscandent shrub with slender branchlets. It resembles *P. niruri* in the origin and nature of its branches, arrangement of leaves, etc. It differs from *P. niruri* in its diffuse and robust habit. The bud in the axil of the leaves of its primary branches develops into a cluster of flowers but never into vegetative branches. During flowering the branch is seen terminated by flower clusters.

Organization of the vegetative shoot apices

The shoot apex of *P. niruri* (Fig. 3) is dome-shaped in resting stage but becomes asymmetrical during the laying down of a leaf primordium. It is occupied by the eumeristem. The outer layer is the typical *tunica* in which the cells divide by anticlinal walls only. Within this tunica layer just behind the extreme tip there is a layer which can easily be differentiated from the rest of the *corpus* cells by their cyto-histological behaviour. At the apex of the dome its cells are vertically elongated and characterized by anticlinal divisions. At the flanks the divisions are by periclinal walls. Both leaf and bud primordia appear to arise in this layer. Even quite early in bud development this layer is seen well organized (Fig. 3 *b*, *d*). In the rest of the corpus cells periclinal, anticlinal and irregular divisions occur. *Flank and rib meristem-like organizations are not noticed.*

The shoot apex organization of the main axis of *P. reticulatus* (Fig. 4) differs to a considerable extent from that of *P. niruri*. The tunica is uni-

seriate but the corpus without any zonal organization, is more extensive and its cell arrangement is most irregular. The outermost layer of the corpus is not organized in the pattern of its counterpart in *P. niruri*, and periclinal divisions are less frequent at the flanks. A desmogen (provascular) strand is seen running up the apex to supply a leaf primordium which is being laid down.

Leaves and their vascular supply

Two types of leaves are found in both the species:

(1) Three-fid scale leaves on the main axis in the axils of which the primary branches arise, and (2) the foliage leaves which the primary branches bear.

The scale leaf and its vascular supply.—(Figs. 5–10)—The mature scale leaf of *P. niruri* is trifid (Fig. 1 *a*). It is supplied by a single trace bundle which causes only one gap in the axial cylinder (Fig. 5). Before entering the free limb of the leaf it divides into three parts, one central and two laterals (Fig. 7). The central enters the middle lobe of the scale and the two laterals, the two lobes of the same (Figs. 8–10). The trace is very feeble and when divides into three parts it becomes feebler, the further growth and development of the scale is therefore much arrested.

Figs. 6, 7, 8, 9 & 10 show a very notable feature which is worth mentioning here. The two bud traces are not branches given out from the two ends of the axial cylinder flanking the gap caused by the departure of the median trace bundle but they are parts of the main stele. Attached to the proximal end of each bud trace is noticed a constricted area which when compared with Fig. 13 (*P. reticulatus*) appears to be the lateral of the leaf trace. It seems that the two laterals instead of departing for the central limb of the scale leaf remain attached to the bud traces. When Figs. 8, 9 and 10 are examined it is further seen that these two constricted portions unite to form the trace of the secondary branch (see below).

The scale leaf of *P. reticulatus* is also trifid. It is supplied by three trace bundles which cause three gaps in the axial cylinder when they depart for the leaf. (Fig. 13). The median like that in *P. niruri* divides but the two lateral branches turn back and unite with the two incoming laterals (Fig. 14), and the central portion goes to supply the scaly leaf. The laterals give out a branch each to supply the lateral lobes with their traces (Fig. 14). The lateral lobes of the three-fid scaly leaf are, therefore, stipules and not the lobes of the scaly leaf (*cf.*, scaly leaf of *P. niruri*, Fig. 7). The composite bundles formed by the union of the lateral leaf traces and the lateral branches

of the median do not enter the leaf but are lost in the leaf-cushion. Fig. 14 shows that the bud traces of the primary branch also remain united with these composite bundles for a short while before the latter are lost (Fig. 15).

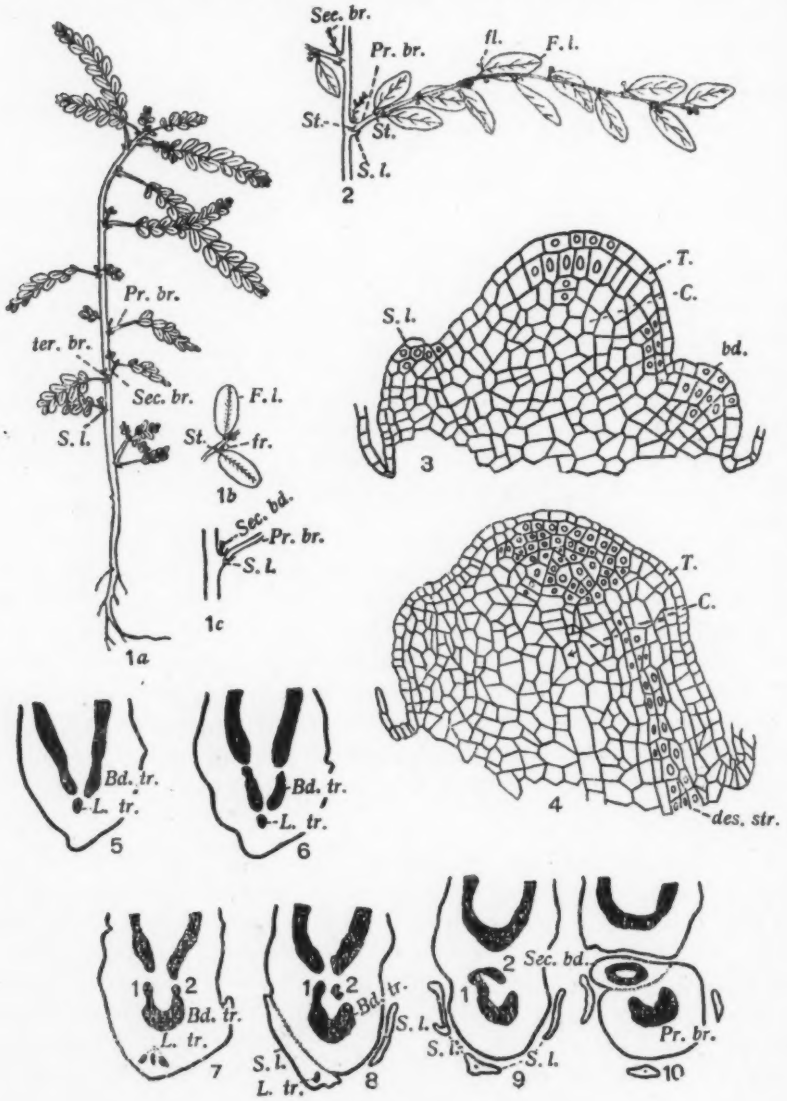


FIG. 1–10

The foliage leaf and its vascular supply.—The foliage leaves of these plants are simple and stipulate. They are borne only on the primary branches which terminate in growing points which are similar in organization to those of the main apices. In *P. niruri* (Fig. 18) these leaves are supplied by three trace bundles. The gap in the central cylinder caused by the departure of the median remains open and the stele in the branch assumes the form of a cup (*cf.*, rachis stele of a compound leaf) (Figs. 20, 21). The cup is later closed and the branch stele forms a cylinder a little beyond the divergence of the first leaf. During their movement towards the median the laterals send out a branch each to form the stipules and their traces on their corresponding sides (Figs. 19, 20).

Foliage leaves of *P. reticulatus* are also supplied by three trace bundles which cause three gaps in the axial cylinder (Figs. 22, 23). The laterals like those in *P. niruri* unite with the median and form an arc in the branch axis (Fig. 24). A branch from each lateral goes to supply the stipules (Figs. 24–26).

Origin of Buds and their Vascular Supply

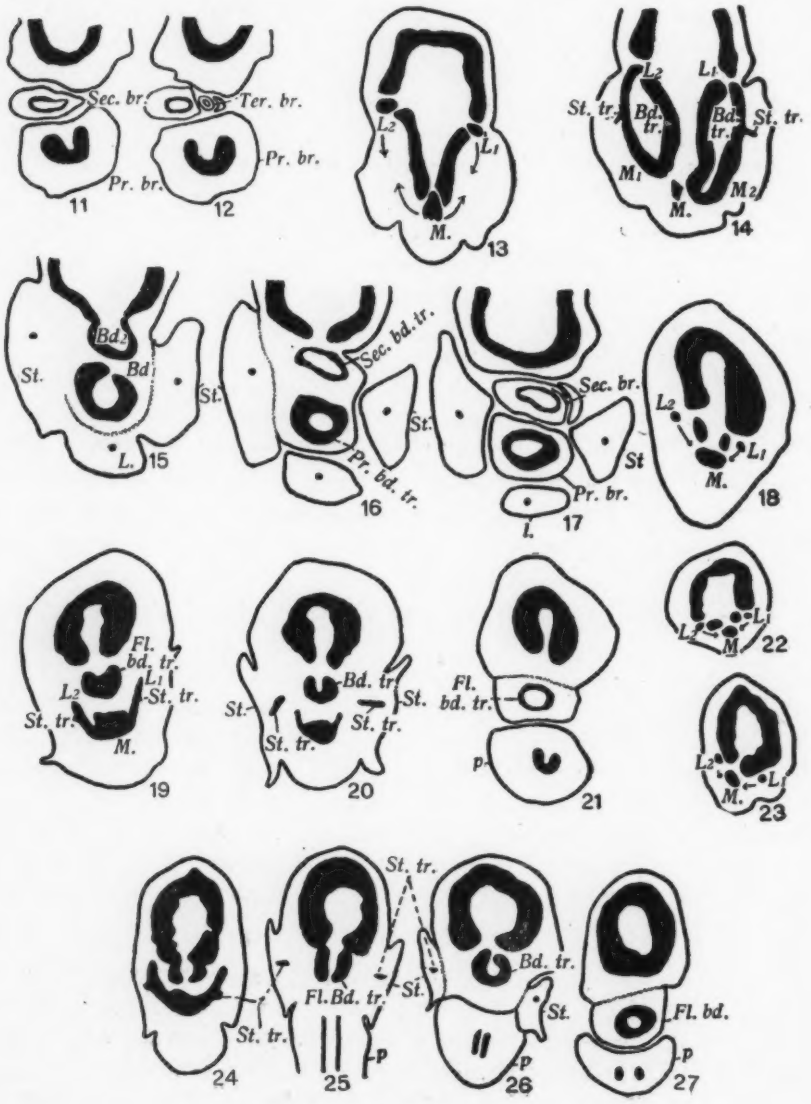
Origin of the primary bud in the axil of the scale-leaf and its vascular supply.—The buds in both the species studied originate in the tissues of the main axis and therefore their origin is *axial* and not foliar.

The first appearance of the bud in *P. niruri* is noticed in relation to the first or second scale leaf from the vegetative apex. The bud traces, two in number, are supplied from the vascular cylinder of the main axis flanking the gap caused by the departure of the median leaf trace bundle (Figs. 5, 6). These two traces run towards the base of the bud, unite by their posterior ends and remain cup-like for about ½–1″ up the axis in mature branches, and then unite by their free ends to form a vascular cylinder (branch stele).

In *P. reticulatus* bud traces are also two in number, but instead of being branches from the free ends of the axial stele flanking the median leaf gap they are its portions between the median and lateral gaps on each side (Figs. 13, 14). Soon after the disappearance of the composite bundles (formed by the union of the laterals and lateral branches of the median) the bud traces unite to form a closed vascular cylinder at the base of the bud axis (Figs. 15, 16).

Origin of the secondary and tertiary buds (*branches*) *in the axil of the primary bud and their vascular supply.*—These buds arise in comparatably mature nodes (Figs. 1 *a* and 2). In *P. niruri* the second and third buds arise in slightly extra-axillary position (Figs. 10–12).

The origin of the two traces of the secondary bud in *P. niruri* as we have indicated before is rather interesting. Figs. 6 and 7 show two constricted areas (1, 2) at the distal ends of the two primary bud traces. They are



FIGS. 11–27

organized immediately after the separation of these traces from the main stele. When compared with Fig. 13 they appear to represent the two laterals of the three-bundle trace of *P. reticulatus*. If that is so then the two laterals of the leaf trace of *P. niruri* (unilacunar node) instead of departing for the median remain tagged to the tail ends of the primary bud traces and ultimately separate from the latter to form the traces of the secondary bud (Figs. 6–9). This appears to be a novel feature in the origin of traces of secondary bud (*cf.*, below for *P. reticulatus*) and further investigation is being carried on with other allied materials to verify the truth or otherwise of the method of origin of secondary bud traces noticed in this species.

The tertiary bud originates lateral to the secondary bud and gets its vascular supply from the axial stele of the latter (Figs. 11, 12). When Figs. 8–12 are examined the sequence of the origin and vascular supplies to the primary, secondary and tertiary buds of *P. niruri* are noticed as follows: The primary bud takes its origin in the tissues of the main axis and gets its vascular supply from the axial cylinder of the same. The secondary bud originates in the tissues of the primary bud and gets its vascular supply from the same, and the tertiary bud in its turn originates and gets its vascular supply from the secondary bud.

The origin of both the primary and secondary buds of *P. reticulatus* takes place in the tissues of the main axis and their vascular supplies are derived from the main stele (Figs. 14–17). *P. reticulatus* thus differs from *P. niruri* in the methods of the origin and vascular supplies to the secondary buds.

Origin of buds (flower) in the axils of the leaves of the primary branches and their vascular supplies.—The buds in both the species are axial in origin. These buds are invariably flower-buds. They generally arise in the axil of the second or third leaf primordium from the tip. In both the species the two bud traces originate from the vascular cylinder of the branch axis from the edges of the gap caused by the departure of the median leaf trace bundle. After separation from the branch stele they immediately unite to form a vascular cylinder and supply the flower bud (Figs. 18–21 and 24–27).

Discussion and Conclusions

The cellular organization at the vegetative shoot apices of both the species studied does not show an advanced type in dicotyledons. In *P. niruri* the tunica is uniseriate but the layer within (outer corpus layer) is rather peculiar in having two regions: the region immediately surmounting the apical dome is made up of vertically elongated prismatic cells bigger than other cells of the eumeristem, and its cells show slow anticlinal divisions;

in the region lower down in the flanks the cells are much smaller in size and divide mainly by periclinal walls. These along with the local tunica cells appear to be concerned in the origin of leaf and bud primordia. The rest of the corpus is a mass of cells without any organization or zonation.

The tunica in *P. reticulatus* is also uniseriate. The corpus in this case is extensive and the arrangement of the cells is most irregular. The desmogen strand is acropetal in its differentiation and appears directly concerned with the erection of the free limb of a leaf primordium on the side of the apical dome. In this case also the zonation of apical meristem is absent.

Cellular organization at the vegetative shoot apices of both the species therefore supports Sporne (1949) according to whom the Euphorbiaceæ to which these two species belong is a primitive family as its advancement index is only 25% (p. 273).

Neither Prain (1903) nor Willis (1951) has described the leaves on the main stem in the axils of which the primary vegetative buds arise. This omission appears to be due to the fact that these leaves are scaly and insignificant in nature. We have seen before that these features are related to their vascular supplies which are very feeble and slender.

The nodes of the main axis in *P. niruri* are unilacunar, and each lateral lobe of the scaly leaf receives its vascular supply from a branch of the median. The lateral lobes are, therefore, parts of the lamina (all the three lobes of the leaf getting their vascular supply from the same median), and are not a pair of stipules.

There are reported cases of a few dicotyledons in which though the nodes are unilacunar the leaves are stipulate. Sinnott and Bailey (1914) and Mitra (1949) who examined and reported on such nodes state that the so-called median bundle in many of them show indications of a composite nature as if formed by the fusion of the two laterals with the median of a three-bundle trace. Our present studies on the nodal anatomy of *P. niruri* give no indication of such fusion.

In *P. reticulatus* the nodes of the main axis are trilacunar and the leaf trace consists of three bundles—the median and the two laterals. The two laterals after giving out a branch each to the two lateral lobes of the scaly leaf disappear in the leaf cushion. The two lobes in the case of this species are, therefore, a pair of stipules (Colomb, 1887; Sinnott and Bailey, 1914; Mitra and Majumdar, 1952, and others).

The above observations on the two species of the Euphorbiaceæ support Sinnott and Bailey (1914) who state that in the Euphorbiaceæ the species

are both uni- and tri-lacunar with exstipulate and stipulate leaves. It is however to be noted that the traces of the leaves on the primary branches in both the species consist of three bundles—one median and two laterals, and the nodes are trilacunar.

P. niruri and *P. reticulatus* belong to the same genus and in great many features resemble each other. The scaly leaf on the main axis are trifid in both the species, but in one case its lateral lobes are *leaf lobes* and in the other species they are *stipu'es*. This distinction has been made only on the basis of their vascularization. A pertinent question may be raised here: Are the *stipu'es* and the pair of basal lobes of a leaf (particularly in these cases) equivalent organs? Sinnott and Bailey (1914) suggested that the stipules may be "considered as homologous with the two basal leaf lobes or the earliest leaf teeth". In the first case the difference is one of degree than of kind, and in the second case their position is determined by the branches of the two lateral leaf traces of a three-bundle leaf trace (p. 451).

Let us examine the suggestions of Sinnott and Bailey in the light of the evidence presented in this paper: The leaves on the primary branches of both the species are simple and stipulate, the leaf trace is made up of three bundles—the median and the two laterals and the nodes are trilacunar. The axillary buds are all flower-buds.

Both the species agree in having scaly trilobed leaves on the main axis and there the common feature ends. In *P. niruri* the leaf trace consists of the median only and the node is unilacular. In *P. reticulatus* on the other hand three bundles, the median and two laterals, constitute the leaf trace and the node is trilacunar. The median in both the species divide into three parts—a central and two lateral branches. In both of them the central portion goes to the central lobe but the behaviour of the two laterals is different in the two species. In *P. niruri* they go to supply the two basal lobes, but in *P. reticulatus* they turn back and join the incoming laterals of the leaf trace which have already sent out branches to the two basal lobes, and finally lost in the leaf cushion. Only on the nature of the vascular supply the basal lobes in one case is described as leaf lobes and in the other case a pair of stipules.

Trilacunar condition of the node and the three-bundle leaf trace go together. Therefore the only difference noticed in the two species is the absence of the laterals in one case and their presence in the other. In the first case the median divides to supply the lateral leaf lobes and in the other case the laterals give out branches to these leaf lobes. On the basis of this difference it may be said that in the present case the difference between the

pair of lateral basal lobes and the pair of stipules " is one of degree than of kind".

Primary buds in both the species arise in the tissues of the main axis and receive their vascular supplies from the main stele (Sterling, 1945; Garrison, 1949 *a*, 1949 *b*; Gifford, 1951; Miller and Wetmore, 1946; White, 1955). The two bud traces in both the cases instead of being branches appear to be portions of the central cylinder between the gaps caused by the departure of the median and the laterals.

The position of the secondary buds in both the species is axillary or slightly extra-axillary to the primary buds. In *P. reticulatus* its origin is in the tissues of the main axis like that of the primary buds, but in *P. niruri* it is in the tissues of the primary bud axis. The vascular supply also appears different in the two species. In *P. niruri* the supply comes from the two free ends of the cup-shaped primary bud trace. Immediately after the separation of the two bud traces from the main cylinder two constricted areas appear at their distal ends. These look like the two laterals of the scale leaf suppressed in this species. These two areas separate from the primary bud traces which meanwhile unite by their proximal ends and form the trace of the secondary bud.

The origin of the secondary bud in *P. reticulatus* is axial (main axis) like that of the primary buds and its vascular supply also comes from the central cylinder. From the very beginning it forms a loop opposite the median gap and after separation from the main cylinder form a closed stele at the base of the bud.

Tertiary buds are extra-axillary in position being situated laterally to the secondary bud, and were found developed only in *P. niruri*. Its origin is in the tissues of the axis of the secondary bud and its vascular supply also comes from the axial stele of the former in the form of a loop as seen in the origin of the trace of the secondary bud in *P. reticulatus*.

The buds in the axils of the leaves on the primary branches develop into flowers without any exception. They get their vascular supply from the stele of the primary branches and not from the stele of the main axis. As the primary branches bear only flower-buds, either singly or in clusters, it is suggested that these branches should be called *inflorescences* and not vegetative branches. The simple stipulate leaves are therefore bracts.

SUMMARY

The apical organizations of the vegetative shoot apices have been studied in *P. niruri* and *P. reticulatus* (Euphorbiaceæ). The uniseriate tunica and

he massive corpus without any zonation in both the species indicate a less advanced apical organization among dicotyledons.

Two types of leaves are borne by both the plants: scaly on the main axis and foliage on the primary branches. The foliage leaves of both the plants are simple and stipulate, and are characterized by a three-bundle leaf trace, trilacunar nodes which bear them, and presence of only flower-buds in their axils. The scale leaves, on the other hand, are three-lobed in both the species, but they differ in the constitution of their traces and nodal anatomy. In *P. niruri* the leaf trace consists of only one bundle, the median, and the node is unilacunar, whereas in *P. reticulatus* the leaf trace is constituted of three bundles and the node is trilacunar.

The two lobes of the scale leaf, on the basis of their vascularization, are described as a pair of basal leaf lobes in *P. niruri*, and a pair of stipules in *P. reticulatus*. The probable homologies of these two structures *in these plants* have been discussed.

The origin and vascularization of the primary, secondary and tertiary buds—the last only in *P. niruri*, have been studied and discussed. The origin of the secondary and tertiary buds and their vascular supplies appear to be novel features not so far as the authors are aware, recorded before.

As the primary branches bear only flower-buds it is suggested that they should be called *inflorescence axes* and the foliage leaves, bracts. This also appears to be a primitive character.

Literature Cited

Colomb, G. .. "Recherches sur les Stipules," *Ann. Sci. Nat.*, 1887, **6** (7), 1.

Garrison, R. .. "Origin and development of axillary buds: *Syringa vulgaris*," *Amer. J. Bot.*, 1949 *a*, **36**, 205.

———— .. "Origin and development of buds: *Betula papyrifera* Marsh and *Euptelea polyandra*," *ibid.*, 1949 *b*, **36**, 379.

Gifford, E. M. .. "Ontogeny of the vegetative axillary bud in *Drimys winteri* var. *chilensis*," *ibid.*, 1951, **38**, 234.

Miller, H. A. and Wetmore, R. H. "Studies in the developmental anatomy of *Phlox Drumondii*—The apices of the mature plant," *ibid.*, 1946, **33**, 1.

Mitra, G. C. .. "Comparative account of the development of the base in the sheathing, the stipulate and the exstipulate leaves of four species of dicotyledons," *Bull. Bot. Soc. Beng.*, 1949, **8**, 33.

——— and Majumdar, G. P. .. "The leaf-base and the internode: their true morphology," *Palæobotanist*, 1952, **1**, 351.

Prain, D. .. *Bengal Plants*, Calcutta, 1903, **2**.

Sinnott, E. W. and Bailey, I. W. "Investigations on the phylogeny of angiosperms. III. Nodal anatomy and the morphology of stipules," *Amer. J. Bot.*, 1914, **1**, 441.

Sporne, K. R. .. "A new approach to the problem of the primitive flower," *New Phyt.*, 1949, **48**, 259.

Sterling, C. .. "Growth and vascular development in shoot apex of *Sequoia sempervirens*," *Amer. J. Bot.*, 1945, **32**, 118.

White, D. J. B. .. "The architecture of the stem apex and the origin and development of the axillary buds in seedlings of *Acer pseudoplatanus*," *Ann. Bot.*, N.S., 1955, **19**, 437.

Willis, J. C. .. *A Dictionary of the Flowering Plants and Ferns*, Cambridge, 1951.

EXPLANATION OF TEXT-FIGURES

FIG. 1 *a*, *b*, *c*. Habit sketch of *Phyllanthus niruri* showing primary, secondary and tertiary branches, scale leaf, foliage leaf, stipules, fruits, etc. 1 *a*, ×½; 1 *b*, ×1; 1 *c*, ×2.

FIG. 2. A portion of *P. reticulatus* showing primary and secondary branches, scale leaf, stipules, foliage leaves, flowers, fruits, etc., ×½.

FIG. 3. Median longitudinal section of the shoot apex of *P. niruri* showing uniseriate tunica, massive corpus, outer corpus layer, bud initiation at the right flank and a scale leaf in development at left, ×562·5.

FIG. 4. Median longitudinal section of the shoot apex of *P. reticulatus* showing uniseriate tunica enclosing extensive corpus, desmogen strand of a new leaf primordium continuous with the eumeristem at its right flank, ×362·5.

FIGS. 5–12. Serial transverse sections through the nodal region of the main axis of *P. niruri* showing unilacunar node, one-bundle (median) leaf trace, origin of traces of the primary, secondary and tertiary buds, vascular supply to the lobes of the scale leaf, etc., ×23·8.

FIGS. 13–17. Serial t.s. through the nodal region of the main axis of *P. reticulatus* showing trilacunar node, three-bundle leaf trace, stipular trace, traces of primary and secondary branches, etc., ×27·5.

FIGS. 18–21. Serial t.s. through the node of a primary branch of *P. niruri* showing trilacunar node, three-bundle leaf trace, stipules with their traces, traces of flower-bud, etc., ×23·8.

FIGS. 22–27. Serial t.s. through the node of a primary branch of *P. reticulatus* showing trilacunar node, three-bundle leaf trace, stipules with their traces, petiole and its trace, flower-bud and its trace, ×23·8.

Pr.br., *Sec.br.* and *ter.br.*, primary, secondary and tertiary branches respectively; *s.l.*, scale leaf; *F.l.*, foliage leaf; *st.*, stipule; *p.*, petiole; *Fl.*, flower; *fr.*, fruit; *bud*, bud primordium; *Fl.bd.tr.*, flower-bud trace; *T.*, tunica; *C.*, corpus; *des.str.*, desmogen strand; *l.tr.*, leaf trace; *Bd.tr.*, bud trace; *M.* and L_1, L_2, median and lateral leaf trace; *l.*, leaf; *St.tr.*, stipular trace; *op.cup.*, open cup-shaped bud trace.

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3

FURTHER CONTRIBUTION TO THE EMBRYOLOGY OF *DROSERA BURMANNI* VAHL.

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(Communicated by Prof. L. Narayana Rao, F.A.SC.)

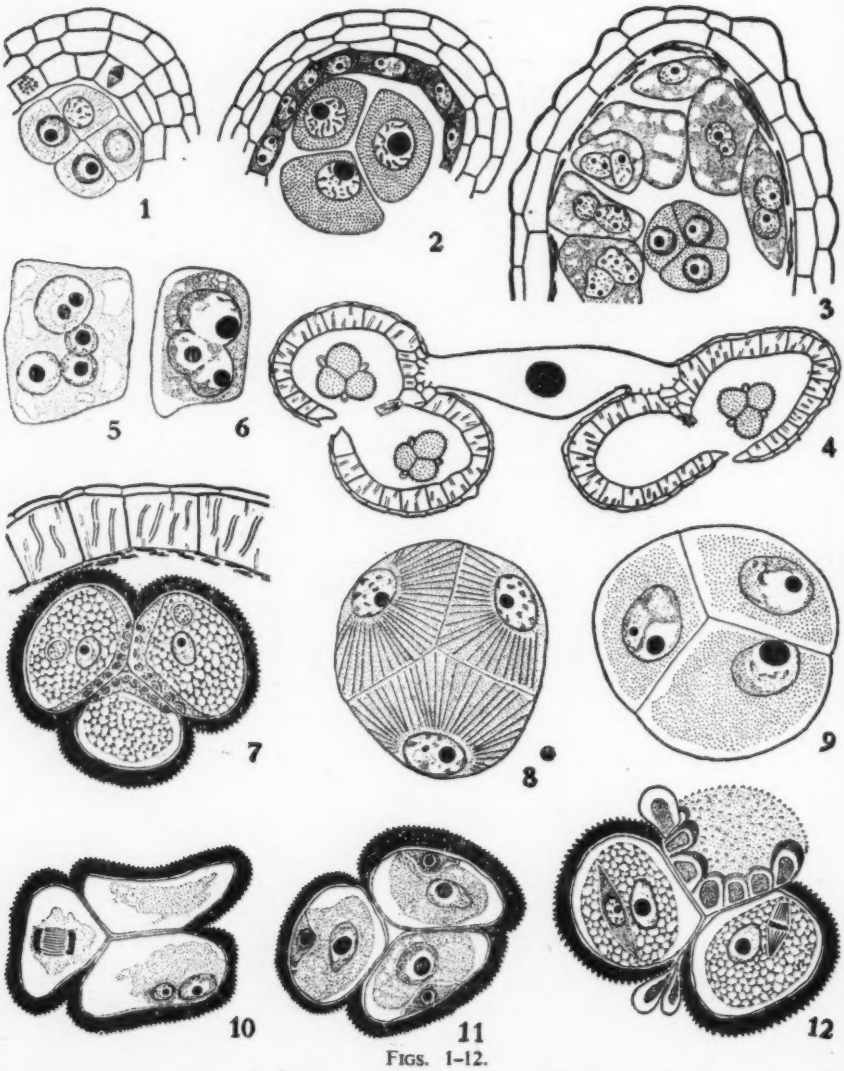
The literature on the embryology of the Droseraceæ has been reviewed briefly by Schnarf (1931). Since 1931 additional information regarding the embryology of some species of *Drosera* has been available from the works of Venkatasubban (1950 *a*; 1950 *b*) and Narasimhachar (1949; 1951). Earlier, Smith (1929) had given an account of some aspects of the embryology of the allied genus *Dionæa*. In his comprehensive account of the seed-coat structure in angiosperms, Netolitzky (1926) has described the seed coat development of some species of *Drosera*. Maheshwari (1955), in his recent review of Bisporic type of embryo sacs in angiosperms, has mentioned the occurrence of a bisporic type of embryo sac in *Drosophyllum*. In view of certain gaps in the account of *Drosera burmanni* by Narasimhachar (1949), a reinvestigation was considered desirable and the observations made are described in this paper.

Microsporangium and Male Gametophyte

The young microsporangium in transverse section is four lobed (Fig. 13). Each lobe has a group of sporogenous cells enveloped by a wall of four layers (Fig. 1) of which the innermost gets differentiated into the tapetum (Fig. 2). During the development of anther the middle layer is crushed (Fig. 3) and the endothecium develops fibrous thickenings (Figs. 4, 7). The epidermis in the mature anther persists and is irregular (Fig. 4). Similar organisation of the anther wall has been reported in *Drosera indica* and *D. peltata* by Venkatasubban (1950 *b*), and in *D. burmanni* by Narasimhachar (1949). The anthers are extrorse and dehisce by longitudinal slits (Fig. 4).

The tapetal cells are usually binucleate but instances of four nucleate cells were also noticed (Fig. 5). The tapetal nuclei may undergo repeated fusions (Fig. 6) and divisions. In *Drosera burmanni* the tapetum is of the glandular type (Fig. 3). But in *D. indica* and *D. peltata* the tapetum is reported to intrude into the loculus (Venkatasubban, 1950 *b*). Further, in the above two species, an indication of tapetal plasmodium has been recorded.

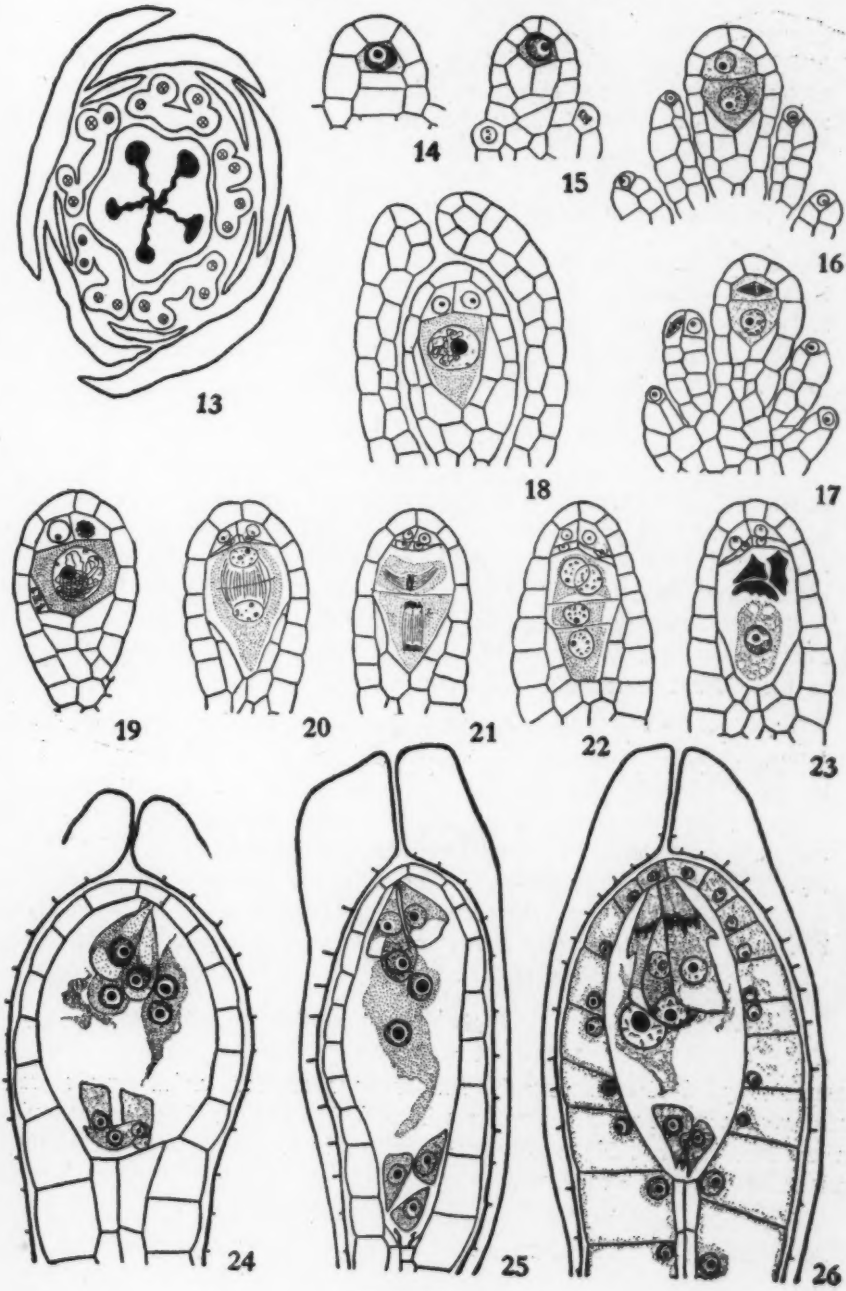
The sporogenous cells (Fig. 1) round off to form microspore mother cells (Fig. 2) and undergo the usual reduction divisions giving microspore



FIGS. 1–12.

tetrads (Figs. 8–12). During the simultaneous division, definite walls are laid down to organise the microspores (Figs. 8, 9) which are always associated in tetrads. Smith (1929) in *Dionæa muscipula* and Erdtman (1943) in *Drosera* spp. have reported tetrads of microspores.

The pollen grain of *Drosera burmanni* has a thick exine and a thin intine (Figs. 7, 10–12). On the distal side characteristic spines of uniform size are



FIGS. 13–26.

developed. But, in *D. indica* and *D. peltata* the spines are large and sparsely distributed (Venkatasubban, 1950 *a*). Such hickenings are not seen on the proximal side. On the other hand, it has a variable number of germinal apertures through which the pollen tubes emerge out during germination (Fig. 12). Very often, even when the pollen tetrads are still in the anther locule the intine protrudes out of the apertures, giving an impression that the grains have germinated *in situ* (Fig. 12).

The microspore nucleus situated in the frothy cytoplasm moves usually towards the distal side and divides to form a small lenticular generative cell and a large tube cell (Figs. 10, 11). Normally in a tetrad the division of the microspore nuclei is not synchronous (Fig. 10). The mature pollen grains of a tetrad may be two or three celled (Figs. 11, 12).

OVARY AND OVULES

The superior ovary is pentacarpellary and unilocular (Fig. 13). The thin ovary wall is about four or five layered and along its inner surface there are five placental protuberances which indicate the pentacarpellary nature in *Drosera burmanni* (Fig. 13). In *D. indica* three and in *D. peltata* two to four placental protuberances have been reported (Venkatasubban, 1950 *b*). In *D. burmanni* four to five placentæ have been shown (Venkatasubban, 1950 *b*; Narasimhachar, 1949). However, such a variation has not been observed in the present investigation of *D. burmanni.*

The parietal placental protuberances bear large number of anatropous bitegmic ovules. The ovular primordium arises as a small outgrowth from the placental tissue (Fig. 14). At about the time of archesporial differentiation the integumental initials are laid down (Fig. 15). Gradually, the ovule takes a bend towards the placenta eventually becoming anatropous. The integuments are two layered each. The outer integument during its growth stops short and does not take part in the organisation of the micropyle (Fig. 27). The inner integument grows over the nucellar epidermis and finally organises the micropyle (Fig. 27).

The primary parietal cell (Fig. 16) derived from the archesporium divides twice to form four cells arranged in a single tier (Figs. 17–23). According to Venkatasubban (1950 *b*) and Narasimhachar (1949) only two cells are present. These in later stages get crushed by the enlarging embryo sac. But, in *D. peltata* and *D. indica* (Venkatasubban, 1950 *b*; Narasimhachar, 1951) no parietal cells have been observed. However, their presence has been reported in many species of *Drosera* (Peters, 1898; Pace, 1912).

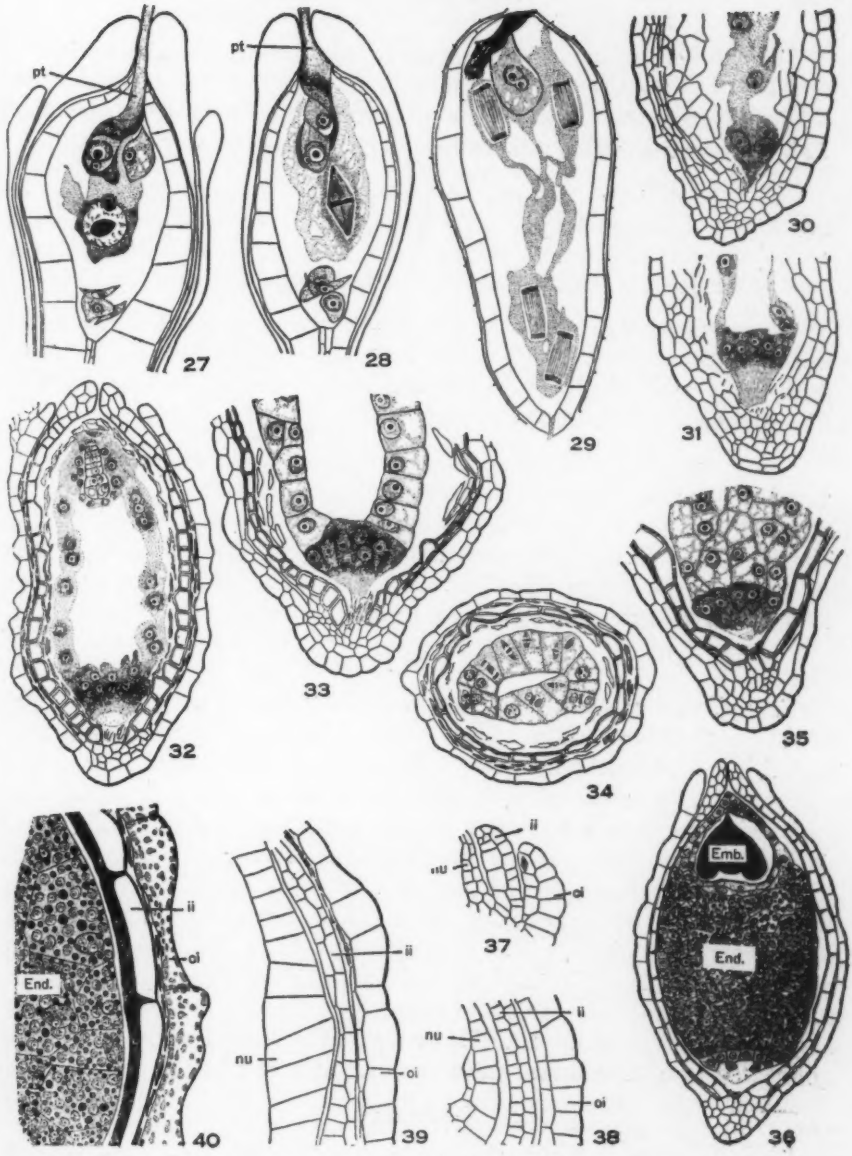

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FIG. 27–40.

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

The present observation on *Drosera burmanni* show that invariably a single archesporial cell is differentiated (Figs. 14, 15). But, Narasimhachar (1949) has figured two and three archesporial cells. The archesporium divides to form a primary parietal cell and the megaspore mother cell.

The megaspore mother cell undergoes reduction division to form a T-shaped tetrad of megaspores (Figs. 19–23). Similar T-shaped tetrad of megaspores have been reported in other species of *Drosera* (Venkatasubban, 1950 *b*; Narasimhachar, 1949, 1951) and in *Dionæa muscipula* (Smith, 1929).

A mature embryo sac of the Polygonum type is organised by the lowermost megaspore (Figs. 23–26). The mode and organisation of the embryo sac are the same in all the investigated species (Venkatasubban, 1050 *b*; Narasimhachar, 1949, 1951; Schnarf, 1931).

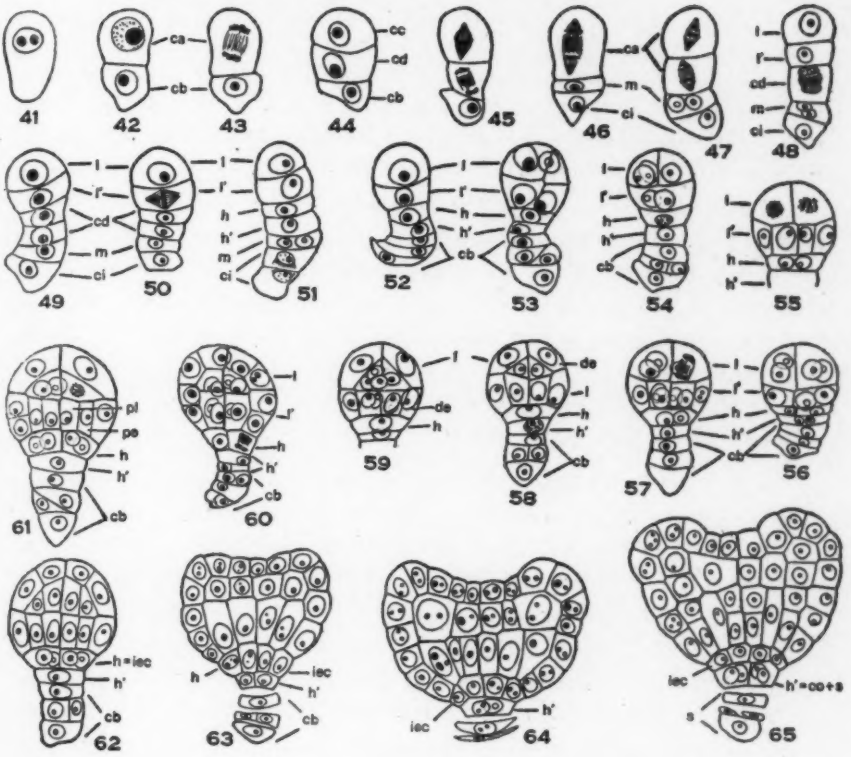
In the embryo sac of *Drosera burmanni* the antipodals are first to organise as cells, followed by synergids and lastly the egg (Fig. 24–26). At the time of fusion of two polars, the synergids are not fully developed (Figs. 24, 25). They, however, show the characteristic vacuolation, hooks and the filiform structures in later stages (Figs. 26). Venkatasubban (1950 *b*) has, however, shown their absence in the synergid cells of *D. burmanni*.

POLLINATION AND FERTILIZATION

The flowers of *Drosera burmanni* are cleistogamous as in other species of the genus (Kerner, 1891; Knuth, 1908). At the time of dehiscence of anthers the stigmatic branches are in close contact with them. The mature pollen tetrads are deposited on the stigmatic branches. Then the pollen tubes from the pollen tetrads germinate into the stylar branches and finally reach the locule of the ovary. One of them enters the micropyle which is organised by the inner integument and reaches the micropylar part of the embryo sac. It penetrates into it by destroying one of the synergids. The other synergid degenerates later. During double fertilization triple fusion precedes syngamy (Figs. 27, 28). Venkatasubban (1950 *b*) has reported that the pollen tube penetrates through one of the synergids and at the time of fertilization both the synergids disappear. However, in the present study one of the synergids is seen to persist for some time even after fertilization (Figs. 27, 28).

ENDOSPERM

The endosperm is free nuclear to begin with (Figs. 28–32) and later on becomes cellular (Figs. 33–36). At about the 32 or 64-nucleate stage the endosperm nuclei gradually move towards the periphery of the embryo sac



FIGS. 41-65.

(Fig. 32). A few nuclei aggregate in the chalazal region and also around the embryo (Fig. 32). The chalzal portion of endosperm with dense cytoplasm penetrates into the adjacent chalazal ovular tissue behaving as a weak haustorium (Figs. 30–32). Gradually, the adjacent ovular tissue is disorganised (Figs. 31–33). Meanwhile, wall formation occurs in the endosperm lying along the sides of the embryo sac (Fig. 33). By subsequent centripetal growth an endosperm tissue is formed (Figs. 34–36). Wall formation in the chalazal region of the endosperm tissue is, however, very much delayed (Fig. 33).

Even after the initiation of cell formation and the formation of an endosperm tissue, the endosperm haustorium in the chalaza remains free nuclear. But, in later stages, between the nuclei thick bands of cytoplasm are laid down (Fig. 33) and ultimately the nuclei become independent by developing walls

(Figs. 35, 36). The rest of the cytoplasm in this region persists in the mature seed (Fig. 36). In the mature seed the persistent endosperm encloses plenty of reserve food materials except at the chalazal region and in some of the cells surrounding the embryo proper (Fig. 36). Similar endospermic seeds with plenty of reserve food material have been observed in *Dionæa muscipula* (Smith, 1929).

EMBRYO

The fertilized egg (Fig. 41) undergoes a transverse division resulting in the apical and basal cells *ca* and *cb* respectively (Fig. 42). The apical cell *ca* generally divides earlier than the basal cell *cb* (Figs. 43, 44). The basal cell *cb* was observed to have divided earlier in a few cases (Fig. 46). Transverse walls are laid down in both the apical and basal cells and a first tetrad of the C_2 category is formed (Figs. 44–47). The cell *cc* by further transverse division results in two cells which are designated as *l* and *l'* (Figs. 48–50). Similarly, the cell *cd* divides transversely giving two superposed cells *h* and *h'* (Figs. 50–54). Thus the four cells *l*, *l'*, *h*, and *h'* derived from cell *ca* constitute the second tetrad. The two cells *l* and *l'* undergo vertical division (Fig. 53). A vertical meridian division in each of these cells results in the two tiers corresponding to the superior and inferior octants (Figs. 56–58).

The four superior octants produce later the cotyledonary part. The inferior octants give rise to the hypocotyledonary part. The first divisions in them separate the dermatogen *de* (Figs. 57–62) and the following divisions the periblem *pe* and the plerome *pl* (Figs. 61–65).

The cell *h* by vertical divisions produces four cells which are distributed circumaxially (Figs. 60, 61) and by further divisions ultimately gives rise to eight cells of which only four are seen in longitudinal section (Figs. 63–65). These four cells adajcent to the axis form the initials of the root epidermis, *iec* (Figs. 63–65) and the peripheral cells contribute to the formation of the lateral part of the root cap (Figs. 64, 65). The cell *h'* divides vertically very late to form two cells (Figs. 63, 64). By one more vertical division the cell *h'* becomes four celled (Fig. 65). Sometimes the cell *h'* divides transversely even before the vertical wall is laid down to form two cells (Fig. 60). The lower daughter cell of *h'* adds to the suspensor.

The two cells *m* and *ci* derived from the basal cell *cb* may undergo a few divisions, mostly transverse and give rise to a short suspensor of a few cells. Often, in one of the suspensor cells a vertical or an oblique wall is laid down.

The mode of development of embryo of *Drosera burmanni* closely corresponds to that described by Souèges (1936) for *D. rotundifolia* and by Narasimhachar (1951) for *D. indica*.

SEED-COAT

The seed-coat in *Drosera burmanni* is organised by both the integuments. To begin with the two integuments are two layered each (Figs. 37, 38). During the development of seed-coat the outer layer of the inner integument and the inner layer of the outer integument get crushed (Figs. 34, 39, 40). The nucellar epidermis persists for a long time, but is later crushed by the developing endosperm (Figs. 36, 40).

The outer layer of the outer integument has longitudinally stretched cells (Fig. 40) which are filled in the later stages with deeply stained substances distributed as globules (Fig. 40). The inner walls of the inner layer of the inner integument are thickened and persist in the mature seed (Fig. 40). Its radial walls are also slightly thickened (Fig. 40). A similar type of seed-coat structure has been described for *Drosera indica* and *D. peltata* (Narasimhachar, 1949, 1951; Venkatasubban, 1950 *b*) and *D. rotundifolia*, *D. intermedia* and *D. anglica* (Netolitzky, 1926).

SUMMARY

The general course of embryology of *Drosera burmanni* agrees with that in the related investigated species.

The anther is quadrilocular and the number of pollen tetrads formed per locule is very much limited. The tapetum is of the glandular type. The mature pollen grains of a tetrad are two or three celled. They are associated in tetrahedral tetrads.

The pentacarpellary ovary has a number of anatropous bitegmic ovules borne on five placental protuberances.

The development of the embryo sac is of the Polygonum type.

The flowers are cleistogamous and fertilization is porogamous. Double fertilization has been observed.

The endosperm is nuclear but later becomes cellular. It persists in the mature seed and encloses plenty of starch and reserve food materials.

The development of the embryo has been followed in detail and is found to resemble closely the development described by Souèges for *D. rotundifolia*.

The seed-coat is organised by the two integuments. In its organisation the inner layer of the inner integument and the outer layer of the outer integument take part.

ACKNOWLEDGMENTS

I am extremely grateful to Professors C. V. Krishna Iyengar and K. N. Narayan, and Dr. M. Anantaswami Rau, Department of Botany, Cen ral College, Bangalore, for their kind interest and helpful suggestions. I am also thankful to Professor S. B. Kausik, for suggesting this problem.

REFERENCES

Erdtman, G. .. *Introduction to Pollen Analysis*, Waltham, Mass, 1943.

Kerner, A. .. *The Natural History of Plants*, London, 1891.

Knuth, P. .. *Handbook of Flower Pollination*, Oxford, 1908.

Maheshwari, S. C. .. "The occurrence of Bisporic Embryo sacs in Angiosperms —A Critical Review," *Phytomorphology*, 1955, **5**, 67-99.

Narasimhachar, S. G. .. "A contribution to the embryology of *Drosera burmanni* Vahl," *Proc. Indian Acad. Sci.*, 1949, **29B**, 98-104.

———— .. "A contribution to the embryology of *Drosera indica*," *ibid.*, 1951, **33B**, 290-95.

Netolitzky, F. .. *Anatomie der Angiospermen-Samen*, Berlin, 1926.

*Pace, L. .. "*Parnassia* and some allied genera," *Bot. Gaz.*, 1912, **54**, 306-29.

*Peters, C. A. .. "Reproductive organs and embryology of *Drosera*," *Proc. American Ass. Adv. Sci.*, 1898, **46**, 275.

Schnarf, K. .. *Vergleichende Embryologie der Angiospermen*, Berlin, 1931.

Smith, C. M. .. "Development of *Dionæa muscipula*. I. Flowers and seed," *Bot. Gaz.*, 1929, **87**, 507-30.

Souèges, R. .. "Embryogenie des Droseraceæ. I. Development de l'embryon chez le' *Drosera rotundifolia*," *C. R. Acad. Sci.*, 1936.

Venkatasubban, K. R. .. "Studies in the Droseraceæ. I. The cytology of *D. indica* Linn., *D. burmanni* Vahl and *D. peltata* Sm. with special reference to pollen mitosis," *Proc. Indian Acad. Sci.* 1950 *a*, **31B**, 308-30.

———— .. "Studies in the Droseraceæ. II. A contribution to the embryology of three species of *Drosera*," *Proc. Indian Acad. Sci.* 1950 *b*, **32B**, 23-38.

* Originals not seen.

EXPLANATION OF FIGURES

FIGS. 1-12. *Drosera burmanni* Vahl. Fig. 1. Portion of anther showing the wall and sporogenous cells; note the division of the innermost wall layer, ×970. Fig. 2. Later stage of the same with three sporogenous cells, the tapetal layer, two middle layers and the epidermis, ×970. Fig. 3. Portion of anther with well differentiated tapetal cells, crushed middle layer, the endothecial layer and the epidermis. One of the micro pore mother cells has divided to form a microspore tetrad, ×970. Fig. 4. T.s. mature anther to show the distribution of fibrous thickenings in the endothecium, and pollen tetrads in the anther locules, ×215. Fig. 5. Tapetal cell with four nuclei, ×1940. Fig. 6. Tapetal cell showing nuclear fusion, ×1940. Fig. 7. Mature anther wall with degenerated middle layer, tapetum and the dwindling epidermis; note the pollen tetrad and the fibrous thickening in the endothecial layer, ×970. Figs. 8-9. Stages of microspore tetrad formation, ×1940. Fig. 10. Sectional view of a pollen tetrad at a later stage to

show the division of the microspore nucleus, ×970. Fig. 11. Sectional view of pollen tetrad at a later stage showing the generative cells at the distal end of the microspores, ×970. Fig. 12. Pollen tetrad showing the generative cell and tube nucleus in one microspore and in the other tube nucleus and the division of the generative nucleus; note the projecting intine from the germ pores, ×970.

Figs. 13–26. *Drosera burmanni* Vahl. Fig. 13. T.s. young flower bud to show the disposition of different floral parts, ×100. Fig. 14. Nucellar primordium with the archesporial cell, ×970. Fig. 15. Later stage of the same with the integumental initials, ×970. Fig. 16. Nucellar primordium showing primary parietal and the megaspore mother cells; note two layered integuments, ×970. Fig. 17–19. Ovule at different stages of development; the parietal cell undergoes division to form four cells, ×970. Figs. 20–22. Different stages of division of the megaspore mother cell during the formation of a tetrad of megaspores, ×970. Fig. 23. Ovule with a tetrad of megaspores in which the micropylar three have degenerated and the fourth chalazal one is enlarging, ×970. Fig. 24. An eight nucleate embryo sac; note the early organisation of antipodals as cells, ×970. Fig. 25. Same at a later stage with three antipodal cells, two free polars, two synergid cells and the egg surrounded by dense cytoplasm, ×970. Fig. 26. Mature embryo sac surrounded by nucellar cells which appear like endothelial cells. In the chalaza the nucellar cells are much elongated and show biseriate condition. Synergid cells show filiform apparatuses and an egg cell lies in between. The secondary nucleus lies towards the egg apparatus. The antipodals are compactly massed together at the chalaza, ×970.

Figs. 27–36. *Drosera burmanni* Vahl. Fig. 27. Embryo sac showing the stage of double fertilization; micropyle is organised by the inner integument, ×647. Fig. 28. Embryo sac with fertilized egg, persistent synergid and antipodal cells and the division of the primary endosperm nucleus, ×647. Fig. 29. Later stage of the same showing division of endosperm nuclei, ×647. Figs. 30–31. Chalazal portion of seed showing the aggregation of endosperm nuclei; note the degeneration of adjacent nucellar cells, ×323. Fig. 32. L.s. young seed with the embryo and endosperm nuclei. In the chalazal region the endosperm nuclei lie in dense cytoplasm, ×287. Fig. 33. Chalazal portion of the seed to show the peripheral cellular endosperm. In the chalazal region the endosperm nuclei are separated by thick cytoplasmic bands, ×323. Fig. 34. T.s. of the same at a later stage showing centripetal growth of endosperm, ×647. Fig. 35. Later stage of the chalazal portion of the seed with the endosperm tissue, ×323. Fig. 36. L.s. mature seed with the embryo and endosperm tissue. The endosperm cells are filled with reserve food materials; but the cells in the extreme chalazal region and also those surrounding the embryo are free from any reserve food materials, ×184. Figs. 37–40. Different stages showing the organisation of seed-coat, ×647. (Fig. 40. A portion of the seed-coat. The outer layer of the outer integument has very much elongated cells with small deeply stained globules. The inner layer of the inner integument has also elongated cells whose inner and radial walls are thickened. Crushed layers between the outer layer of the outer integument and the inner layer of the inner integument could be made out. The endosperm cells are filled with rich reserve food materials. The endosperm tissue closely abuts the inner layer of the inner integument). (*emb*, embryo; *end*, endosperm; *ii*, inner integument; *nu*, nucellus; *oi*, outer integument; *pt*, pollen tube).

Figs. 41–65. *Drosera burmanni* Vahl. Figs. 41–64. Stages in the development of embryo, ×647. Fig. 65. Mature embryo showing the cotyledonary and stem-tip portions; note the dislodged suspensor cells, ×647. (*ca* and *cb*, apical and basal cells, being the daughter cells of fertilized egg; *cc* and *cd*, superior and inferior cells of the apical cell; *ci*, basal cell of a tetrad; *co*, root cap; *de*, dermatogen; *h*, and *h'*, daughter cells of *cd*; *iec*, initials of the root epidermis; *l* and *l'*, superior and inferior octants; *m*, intermediate cell of a tetrad; *pe*, periblem; *pl*, plerome; *s*, suspensor).

ANTHASTHOOPA, A NEW GENUS OF THE SPHÆROPSIDALES

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Received January 13, 1956

An interesting pycnidial fungus was collected on dead pods of *Cæsalpinia pulcherrima* Swartz., from the University Botany Laboratory Garden, Madras. A description of this fungus is given below.

The fungus forms numerous pycnidia all over the dead pods. The pycnidia appear as minute, pin-point-like, somewhat circular, elevations on the surface of the pod and are separate. The pycnidia are immersed in the substratum and are depressed-globose. The pycnidial wall is membranous and is composed of 2–4 layers of pseudoparenchymatous, dark brown cells. There is no evidence of a morphologically differentiated ostiole and dehiscence appears to be due to the bursting of the top of the pycnidium through the tissue of the substratum. The conidiophores are not produced from all over the inner surface of the pycnidial wall, but are produced entirely from over a hemispherical mound of fungal tissue, simulating a sporodochium. The cells of this tissue are minute and subhyaline to pale brown. The conidia are one-celled, hyaline, dorsiventral, and are produced acrogenously and singly at the tips of simple, subhyaline conidiophores. Each conidium has an apical, hyaline, mucoid appendage which is whip-like, the whip being turned backwards and lying parallel and closely touching the concave side of the conidium. This appendage is as long as the spore itself or sometimes slightly shorter.

In the possession of a mucoid apical appendage, the fungus resembles the genus *Neottiospora* Desm. (Subramanian and Ramakrishnan, 1953). However, the appendage in the present fungus is not funnel-shaped as in *Neottiospora*, but is solid and whip-like, and is always turned back and lies closely appressed to the concave side of the dorsiventral spore. A further noteworthy feature of our fungus is the sporodochium-like mound of tissue, at the base of the pycnidial cavity, from which the conidiophores arise. So far as we are aware, no genus of the Sphæropsidales combining the characteristics of this fungus has been described so far and we are, therefore, proposing a new genus to accommodate it. The generic and specific names are both derived from Sanskrit: the generic name from *antha* (अन्त) = internal, and

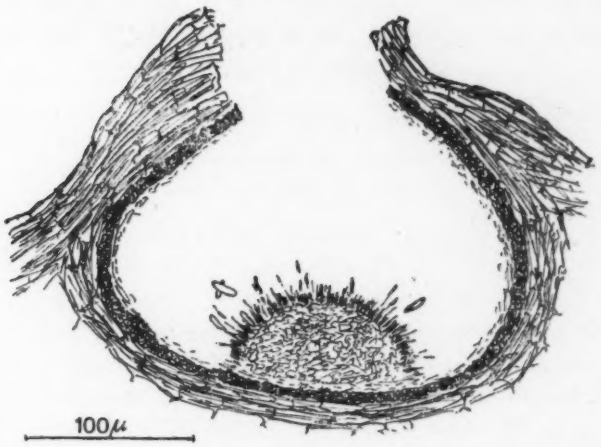

FIG. 1

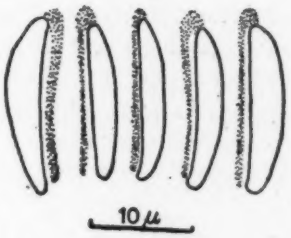

FIG. 2

FIGS. 1–2. *Anthasthoopa simba* from type specimen, Herb. M.U.B.L. No. 808. Fig. 1. Longitudinal section of pycnidium (diagrammatic). Fig. 2. Conidia.

sthoopa (स्तूप) = mound, column; and the specific name from *simba* (सिंब) = legume, suggestive of the substratum on which the fungus occurred.

Anthasthoopa SUBRAMANIAN AND RAMAKRISHNAN GEN. NOV.

Pertinet ad Fungos Imperfectos, ad Sphæropsidales, Sphæroideas, Hyalosporas. Pycnidia immersa, parietibus membranaceis, absque stromate. Conidiophori producti ex cumulo textuum sporodochii similium ad basim cavitatis pycnidialis. Conidia semel cellulata, hyalina, dorsiventralia, producta singulariter atque acrogene, singula ornata appendice apicali, hyalina, mucoidea retrorsum versa, intime adpressa atque parallela lateri concavo conidii.

Fungus imperfectus, Sphæropsidales, Sphærioideae, Hyalosporæ. Pycnidia immersed, with membranous wall, without stroma; conidiophores

produced from the surface of a sporodochium-like mound of tissue at the base of the pycnidial cavity. Conidia one-celled, hyaline, dorsiventral, produced singly and acrogenously, and each having an apical, hyaline, mucoid appendage turned backwards and lying closely adpressed and parallel to the concave side of the conidium.

TYPE SPECIES: **Anthasthcopa simba** SUBRAMANIAN AND RAMAKRISHNAN SP. NOV.

Pycnidia minuta, circularia, immersa, depresso-globosa, tum erumpentia, dehiscentia per rupturam apicia; parietes pycnidiales membranacei, constantes e 2–4 seriebus cellularum brunneolarum atque pseudoparenchymaticarum. Conidiophori simplices, hyalini, surgentes e cumulo textus fungalis sporodochio similis ad basim cavitatis pycnidialis. Conidia semel cellulata, hyalina, fusiformia, tenuiter curvata atque dorsiventralia, producta acrogene atque singulariter ad apices conidiophororum, singula ornata appendice apicali, hyalina, mucoidea, flagelliformi, retrorsum versa atque parallela et intime adpressa lateri concavo conidii; conidia 15–19 × 2·5–3·4 μ; appendix aequilonga conidio, vel eo tenuiter brevior.

Typus lectus in leguminibus emortuis *Cæsalpiniæ pulcherrimæ* Sw. in campo laboratorii botanici Universitatis, in urbe Madras, die 26 mensis Junii anni 1952 a C. V. Subramanian, et positus in herbario M.U.B.L. sub numero 808.

We thank Professor T. S. Sadasivan for much encouragement, the Rev. Father Dr. H. Santapau for kindly translating the diagnoses into Latin and P.of. V. Raghavan for suggesting the generic and specific names in Sanskrit.

REFERENCES

Subramanian, C. V. and Ramakrishnan, K. "On the nature of the spore appendage in *Neottiospora* Desm.," *Proc. Indian Acad. Sci.*, 1953, **37 B**, 228–31.

ON *NEOTTIOSPORA COPROPHILA* SPEG.

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Received January 13, 1956

Neottiospora coprophila was described by Spegazzini in 1879 from a collection made in northern Italy on dung of sheep. A description of this fungus, from Saccardo (1884, p. 217) is given below: " Peritheciis minutissimis, 80–70, globosis, astomis (?), irregulariter dehiscentibus, contextu densiusculo, parenchymatico-indistincto; sporulis oblongo-fusoideis, 25 × 3, utrinque acutiusculis, nubiloso-granuloso-farctis, hyalinis, sessilibus, apice tribus rostellis exilissimis, 20 × 1, ornatis. *Hab.* in fimo ovino vetusto in pratis circa Conegliano Italiæ bor., socia *Delitschia Winteri.*"

Through the kindness of Dr. J. C. Lindquist of the Instituto de Botanica " Spegazzini ", University of La Plata, Argentina, we have been able to examine what is presumably the type specimen of this fungus. The cover containing the material received from Dr. Lindquist is labelled simply " Neottiospora Sporormium status conidicus socia Delitschia Winteri 10–7–1878 " in Spegazzini's handwriting and also contains a rough sketch of the pycnidium with the words " contextu parench. indist." and sketches of three conidia with the words " hyal. 20 × ½, 25 × 2 ". 20 × ½ refers obviously to the measurement of the spore appendage and 25 × 2 to that of the spore itself, both in microns. The writing and the drawings are all in pencil. In sending us the specimen Dr. Lindquist stated: " ....I enclose herewith the specimen that I suppose is the type, because as you will see in the original envelope Spegazzini had marked neither the species nor the type, but this occurs in some Italian Spegazzini's collections. The only sign that permits infer this is given by the date of collection and that like the original publication says: socia *Delitschia Winteri*". A further reason to suppose that the material represents the type is the fact that the spore and appendage measurements given in the envelope match fairly well with Spegazzini's description and the words " parench. indist." given in the envelope appears in the description also. We, therefore, presume that the material received from Dr. Lindquist is the type. We may add that, so far as we are aware, type material of this fungus is not available elsewhere.

The material consists of small fragments of dung which have become powdery and no fungus could be discerned in the powdery material. However, just one pellet of dung, about 0·5 cm. in diameter, wrapped in a small

bit of paper separately, showed a number of minute, black pycnidia on the surface of the pellet. Detailed study of the structure of the pycnidia has not been possible because of the meagre material available, but examination of scrapings of one pycnidium indicated that the pycnidial wall is membranous and is made up of brownish pseudo-parenchymatous cells. The scrapings of the material showed an abundance of pycnidiospores. These spores are hyaline, sub-cylindrical, broader towards the apex and narrowed towards the base which is blunt and rounded, and 18–21 × 1·6 μ. Each spore has 3 or 4 filiform, hyaline, divergent appendages arising from all round the

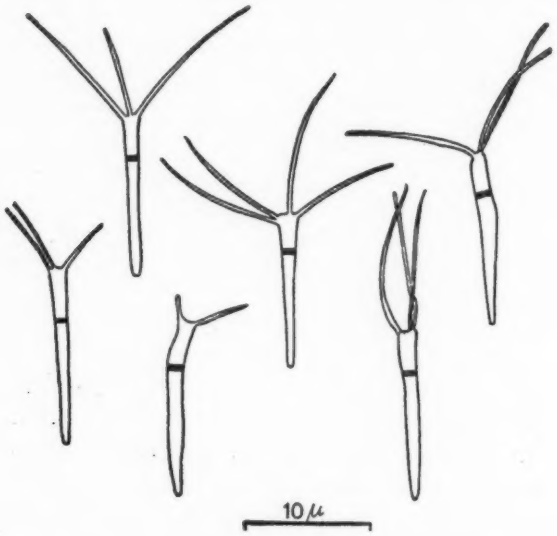

FIG. 1. Pycnidiospores of *Robillarda coprophila* from type specimen, Herb. M.U.B.L. No. 1504.

flattened apex of the conidium. The appendages are 14–16 μ long. However, the spores are not one-celled as stated by Spegazzini, but each spore has a clear septum dividing it into two unequal cells of which the lower one is almost twice as long as the upper one. Thus, the fungus belongs to the hyalodidymæ of the Sphæropsidales-Sphærioideae and not to the hyalosporae of this group, as described by Spegazzini and figured by him in the packet containing the type specimen. Further, the appendages are persistent and not mucoid and are, therefore, unlike those of *Neottiospora caricina* (Desm.) Hoehnel, the type species of *Neottiospora* (Subramanian and Ramakrishnan, 1953). Indeed, Diedicke and Hoehnel have already suggested that

N. coprophila is not a *Neottiospora* (Hoehnel, 1924), although they did not assign the fungus to any other genus.

Since *Neottiospora coprophila* has hyaline didymospores with apical appendages which are persistent and non-mucoid, it cannot be retained in the genus *Neottiospora* Desm. It agrees essentially with *Robillarda* Sacc. (Saccardo, 1884, p. 407) and since it differs sufficiently both in habitat and in spore characteristics from species of this genus so far known, we propose to transfer it to this genus as a separate species:

Robillarda coprophila (SPEG.) SUBRAMANIAN AND RAMAKRISHNAN COMB. NOV.

Basonym: *Neottiospora coprophila* Speg., 1879, *Michelia*, **1**, 481; Saccardo, P. A., 1884, *Sylloge Fungorum*, **3**, 217.

Type (?) : Herb. M.U.B.L. No. 1504, in fimo ovino, Italia, 10–7–1878, leg. C. Spegazzini. Socia: *Delitschia Winteri*, ex Colecciones Micologicas Universidad Nacional de la ciudad Eva Peron Museo-Instituto Spegazzini No. 11564.

We are grateful to Dr. J. C. Lindquist and Dr. A. E. Jenkins for making available the type material of *Neottiospora coprophila*. We are also indebted to Professor T. S. Sadasivan for much encouragement.

REFERENCES

Hoehnel, von F. .. "Ueber die Gattung *Neottiospora* Desm.," *Mitt. bot. Lab. tech. Hochsch. Wien*, 1924, **1**, 78–85.

Saccardo, P. A. .. *Sylloge Fungorum*, 1884, **3**, 860 (Litho-printed by Edwards Brothers, Ann Arbor, Michigan, 1944).

Subramanian, C. V. and Ramakrishnan, K. "On the nature of the spore appendage in *Neottiospora* Desm.," *Proc. Indian Acad. Sci.*, 1953, **37 B**, 228–31.

THE LIFE-HISTORY OF A BRACKISH WATER AMPHIPOD *GRANDIDIERELLA BONNIERI* STEBBING*

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Introduction

Our knowledge of the life-history of amphipods is derived from the accounts given by Sexton and Mathews (1913) and Sexton (1924). Sexton (1924) has stated: "Many of the difficulties found in naming and classifying crustacea are due to the extraordinary modifications caused by growth and sex, and the confusion arising from our lack of knowledge of these developmental stages has led to many errors." This is found very true in the case *Grandidierella bonnieri* Stebbing, for some stages of this species, referred to by Chilton (1921) as adult stages, were later pointed out by Barnard (1935) to be altogether of a different species. This shows that the confusion as to the proper determination of species has arisen from lack of our knowledge about the life-histories of the animals. This work was started in the hope that it might throw some light on such problems.

Material and Methods

Grandidierella bonnieri Stebbing, is a very common species, found in large numbers attached to green algæ at Cooum and Adyar rivers which are shut off from the sea by a bar of sand for nearly eight months during the year. With the onset of the rainy season the bar is opened and there is a great lowering of the salinity of the water. These amphipods are able to withstand sudden changes of concentration as well as temperature of the medium. They are reared in the laboratory without any difficulty and fresh supplies are obtainable easily from the neighbourhood.

A feature of difference between the natural habitat and surroundings and that offered in the laboratory must be mentioned. In the natural surroundings, the amphipods when they moult, seek the shelter of floating weeds, debris and stones. In the laboratory, to facilitate observation, the organisms

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were left in clear glass bowls with water, in bright light with a few strands of algæ intended more as food rather than as shelter. It is therefore probable that some of the observations on the incidence and duration of moulting may not be the same as what is obtained in nature. It was found much easier to study the moults rather than the live animals which soon after taken in a slide for microscopical examination gave violent jerks and tried to move away from the transmitted light. The study of the successive moulting stages was hence carried out by examining the moults, of the same individual as far as possible.

A collection of this species *Grandidierella bonnieri* was made on 6th December 1948 from the river Cooum and brought to the laboratory along with some algæ. Three females with brood pouches in a fully developed condition were picked out by means of a pipette and each one was put in a separate finger bowl containing brackish water. One or two small strands of algæ were also put in each bowl as food for the animals. These finger bowls were marked A, B and C.

Mating and Bearing Eggs

The female in finger bowl A when examined on the morning of 7th December 1948 was found to have its brood pouch empty having liberated twenty young ones which were moving in the bowl. The young ones were removed from the bowl. The next day the animal had moulted and the moult was taken out and a male was also added on to the same container and when observed on the morning of 9th December it was found that the brood pouch contained a fresh batch of eggs. The mating most probably must have occurred at night and as such was not observed. Daily observations continued as usual and on 14th December 16 young ones were liberated from the brood pouch. Three young ones were taken and left in three separate finger bowls for further observations and study (A_1, A_2 and A_3). Observations made on these animals are given in Table I.

The female in B also when examined on the morning of 7th December was found to have its brood pouch empty and 17 young ones were found moving in the bowl. The female was transferred to another bowl along with a male. The next day the female was found to have moulted and on 10th December it was found carrying a fresh batch of eggs in the brood pouch. 23 young ones were seen swimming about in the bowl on the 15th December. Three young ones, B_1, B_2 and B_3 were taken and put in 3 different bowls for the study of the moulting stages (Table I).

On 8th December the female in bowl C was found to have liberated 14 young ones. They were all removed and a male was added and on 10th

TABLE I

	Date on which young one was liberated from brood pouch	Period in days between moults 1 2 3 4 5 6 7 8	Date of reaching adult stage (brood pouch with eggs)	Date on which the next generation was hatched	No. of young ones of the next generation
A_1	14–12–1948	3 2 2 3 4 8 7 7	13–1–1949	19–1–1949	15
A_2	14–12–1948	3 3 2 4 4 7 5 6	11–1–1949	17–1–1949	19
A_3	14–12–1948	2 2 3 4 5 4 7 6	Male	..	..
B_1	15–12–1948	4 2 2 4 5 6 5 5	12–1–1949	17–1–1949	22
B_2	15–12–1948	3 3 4 3 5 7 5 5	14–1–1949	19–1–1949	17
B_3	15–12–1948	3 2 3 4 3 4 5 7	18–1–1949	15–1–1949	19
C_1	18–12–1948	2 2 4 4 5 3 5 8	Male	..	..
C_2	18–12–1948	3 2 3 4 4 5 4 6	Male	..	..
C_3	18–12–1948	3 2 3 4 4 5 4 6	18–1–1949	22–1–1949	20

December it was found that both male and female had moulted and on 11th December the brood pouch contained a fresh batch of eggs. On 18th December 17 young ones came out of the brood pouch. Three young ones, C_1, C_2 and C_3, were placed in 3 different bowls in the same way as before for further observation (Table I).

Sexton (1924) has observed that *Gammarus locusta* lay eggs without a male being present but the eggs were infertile and thrown off later. She has also stated that *Gammarus chevreuxi* and *Gammarus pulex* were not found to lay eggs without the addition of a male. In the case of *Grandidierella bonnieri* it has been observed that females left without males in finger bowls never laid eggs.

BROOD POUCH

The brood pouches are formed by 4 pairs of brood plates, (lamellæ) one pair each on the peræon segments 2, 3, 4 and 5 in the female amphipod. These plates at their margins are fringed with long and thin hairs, which interlace and form a continuous pouch, open at both ends where the plates are separated by the width of the body. The young ones remain in the brood pouch of the mother for a day or two before they are liberated. The newly hatched young ones are very active and move about inside the pouch and try to push open when the mother relaxes the pressure on the brood plates. Through the openings on either ends of the brood pouch the young ones try to come out but the mother can close the entrance at will by bending down

her projecting mouth parts and by lateral compression with her gnathopods and peræopods. The young ones at times crawl on the back of the pouch, but the first beat of the pleopods forwards drives them back safely into the pouch.

The Process of Moulting

In *Grandidierella bonnieri* the process of moulting has been observed only in two instances. The head portion with the antennæ and mouth organs attached is pulled off first. Then the body with the peræopod is drawn out of the posterior part, and lastly the gnathopods are pulled out. In a good moult the skin comes off whole except for the break through which the body is pulled out. The side plate attached to the first joint of each peræopod slips down its internal face. A semicircular flap of skin opens at the place of insertion of the second joint and six free tapering joints are easily drawn out through this opening, leaving the old cuticle intact. In difficult moults the old skin of the gnathopods is torn to pieces. Often the mouth organs are used in the act of stripping it off. Since the skin on the gills is much thinner and flimsier than on any other part of the body, it always gets crumpled or torn in moulting. This happens also in the brood plates in the immature female.

In this connection the difficulties in getting a series of moults from the same animal may be mentioned. Quite a large number of moults were taken but none of the series was complete. The amount of chitin deposited during the animals life varies with individuals, some moults therefore being fairly thick and easily sloughed off, while others thin in texture crumbling off when cast. The chief difficulty also lies in the fact that the animals eat their moults soon after they are cast. In most cases it was observed that the moulting took place at night, and if it had happened early in the night, there was very little chance of obtaining the moult.

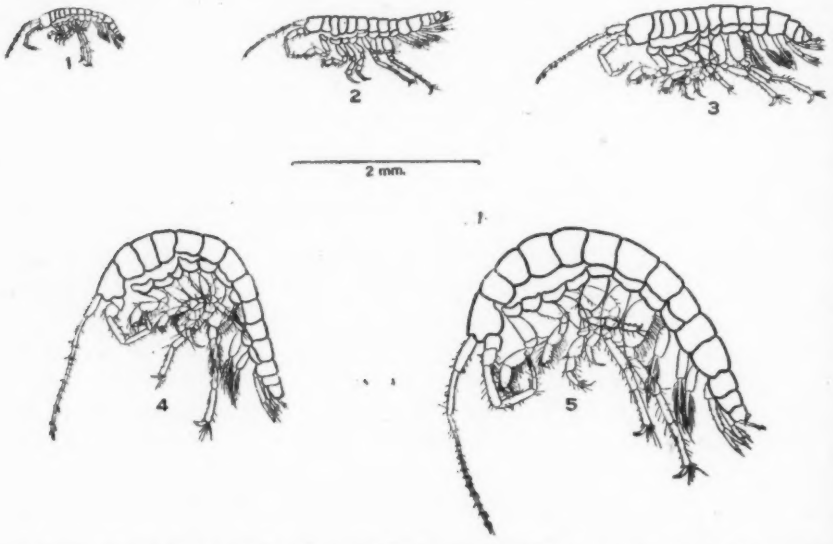
Developmental Stages

The young animal soon after it is hatched out resembles the parents in having all the appendages present. The differences in the proportion of the various parts of the body is however, very marked.

Stage 1 (Fig. 1).—The characters distinguishing this stage are: primary flagellum of antenna 1, 4 jointed; gnathopod 1, palm not well differentiated and uropod 3, with 2 spines at the distal end of the ramus.

The head is fairly large when compared to the body and is as long as the first 2 segments combined. Antenna 1, the peduncle slightly longer than the flagellum; flagellum 4 jointed; first joint of the peduncle stout, shorter

than the second with 2 or 3 small setæ on the lower side; second joint with one small seta each at the distal end on either side; third joint shorter than the second with 2 or 3 setæ at its distal end on either side and also a small accessory flagellum rather very small with 2 setæ at its tip; first joint of the flagellum devoid of any setæ at its distal end; second joint with one spine on either side at its distal end; third and fourth with a sensory filament each at its lower side in addition to the 2 or 3 setæ present at the distal ends of the joints. At the tip of the fourth joint there arises another very short joint (which is not counted in any of these cases) with 2 or 3 setæ. Antenna 2, flagellum shorter than the peduncle; flagellum 3 jointed; joints provided with small setæ both on the inner and outer margins and the last



FIGS. 1–5. Growth-stages of *Grandidierella bonnieri*. Fig. 1. Stage 1. Fig. 2. Stage 2. Fig. 3. Stage 3. Fig. 4. Stage 4. Fig. 5. Stage 5. (The gills and lamellae are omitted from the drawings to avoid confusion of detail.)

joint of the flagellum with 4 to 6 short setæ at its tip. Gnathopod 1, fifth joint as long as sixth; fifth joint with 4 spines on the inner margin and 2 at the distal end of the outer margin; sixth joint with 3 spines on the inner side and a single long spine at the middle of the joint at the outer side and 3 small setæ at the distal end; seventh joint curved, inner side with 2 or 3 small spines. Gnathopod 2, fifth joint nearly as long as sixth; broader towards the base, 4–5 setæ at the inner distal end 2 spines at the outer distal end; sixth joint, palm rather defined by a sharp angle and also provided with one spine

at the defining angle; seventh joint curved reaching up to the end of the palm; inner side serrated. Peræopods 1 and 2 almost the same; joints provided with one seta each at either side at the distal end of each joint; seventh joint long nearly as long as sixth. Uropod 1, peduncle slightly longer than the rami; rami provided with 4–6 small spines at its distal end; peduncle with a small spine at the upper distal end; both the rami devoid of any setæ or spines on inner and outer margins. Uropod 2, very much like uropod 1, but much smaller. Uropod 3, uniramous; peduncle shorter than the ramus; ramus with 2 spines one small and the other long; peduncle as well as the ramus devoid of spines on both the margins. Length of the animal from front of the head to the end of uropods is about 1 mm.

Stage 2 (Fig. 2).—Head longer than the first two segments combined. Antenna 1, peduncle shorter than the flagellum; first joint of the peduncle with 2 small spines at the distal end; flagellum 6 jointed with sensory filaments on fifth and sixth joints. Antenna 2, flagellum short, 3-jointed; one curved spine at the distal end of second joint and two at the distal end of the third joint. Gnathopod 1, fifth joint smaller than sixth; lower margin with 6 spines; sixth joint with 5 spines at the inner margin. Gnathopod 2, fifth joint with lower margin slightly bulged out at the middle; sixth joint with 2 small spines at the middle of the outer margin and 3 spines at the outer distal end. Uropod 1, peduncle with a single spine at the distal end; rami equal; devoid of spines on the inner and outer margins; one long and 3 short spines at the tip. Uropod 2, like uropod 1, but smaller. Uropod 3, rami with a single long spine at the tip and a smaller one just behind it. Length of the animal is about 1·5 mm.

Stage 3 (Fig. 3).—Antenna 1, first joint of the peduncle with 2 small spines at the distal end; flagellum 8 jointed with sensory filaments on fifth, seventh and eighth joints. Gnathopod 1, second and third joints with a single spine each at the lower distal end; fifth joint slightly smaller than the sixth, lower margin with 4 or 5 setæ and one or two spines; sixth joint with a stout spine at the middle of the lower margin. Gnathopod 2, second joint with 2 setæ on the upper margin and one small spine at the inner distal end; fifth and sixth joints subequal; palm differentiated with a small spine at the angle; sixth joint widening distally. Peræopods 1 and 2 alike; second joint with 3 small spines each on the upper and lower margins; fourth joint with a long slender spine at the anterior distal end and a short one at the middle; fifth joint with 2 small and 1 long spine at the lower margin; sixth joint slightly longer than fifth; seventh as long as the preceding joint. Uropod 1, peduncle longer than the rami, peduncle with 3 spines on the upper margin and one on the lower margin. Both inner and outer ramus with a single spine each

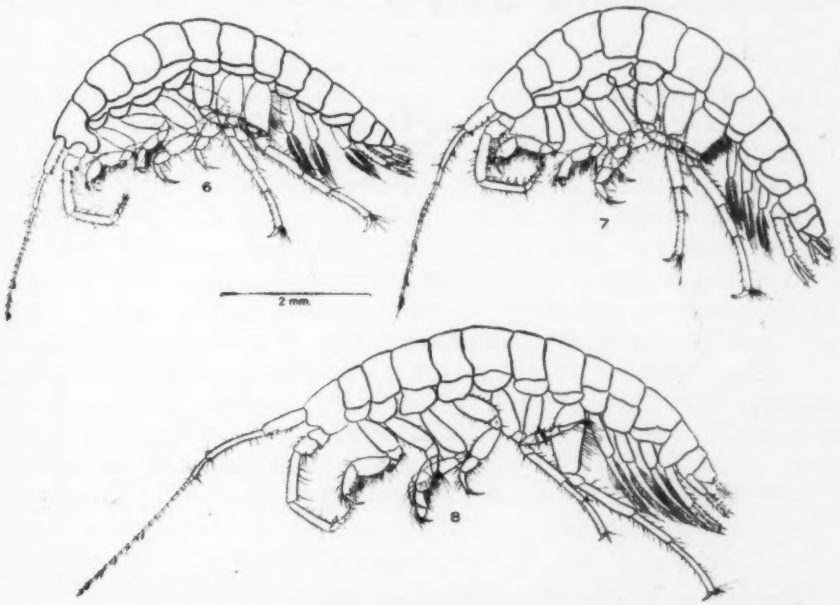
on the upper margin at the middle and the usual terminal spines at the end. Uropod 2, outer ramus without any spines on the margins and with 3 spines at the distal end. Inner ramus with a small spine on the upper margin. Uropod 3, both the margins of the ramus, devoid of any spines; distal end with 3 or 4 spines. Length of the animal is about 2 mm.

Stage 4 (Fig. 4).—Head nearly as long as the first 2 segments combined. Antenna 1, first joint of the flagellum with 5 spines at the lower margin; 4 or 5 small setæ at the middle and 2–4 setæ at the upper side; accessory flagellum rudimentary with 2 small setæ at its tip; flagellum 10 jointed. Antenna 2, peduncle much longer than the flagellum; fourth and fifth joints of the peduncle subequal; flagellum smaller than the penultimate joint. Gnathopod 1, fifth joint longer than sixth; fifth with 7–8 spines at the lower margins; outer margin with one or two distal spines; sixth joint lower margin slightly bulged at the middle with a well-developed single spine and 4–6 small setæ. Gnathodpod 2, fifth joint with 7–8 spines at the inner margin; sixth joint palm curved slightly with a few setæ at the margins. Peræopods 1 and 2 alike, second joint rather long; third joint slightly longer than fifth with the outer distal end produced; seventh joint tapering and slightly longer than the preceding joints. Uropod 1, peduncle with 2 spines on the upper side on both inner and outer margins; outer ramus with 2 spines on the outer margin and inner ramus with 1 spine on the inner margin. Uropod 2, with one spine each on the upper margins of each ramus. Uropod 3, with a single spine on the upper margin, at the middle and 4 spines at the distal end. Length of the animal is about 3–4 mm.

Stage 5 (Fig. 5).—The sexual differentiation is noticed at this stage. Antenna 1, the lower side of first joint of the peduncle with a single spine at the distal end and 2 short spines behind it; two more lateral spines on the lower side; second joint of the peduncle with 4–6 small setæ on the lower side and 6–8 setæ on the upper; flagellum 12 jointed with sensory filaments on seventh, ninth, eleventh and twelfth joints. Antenna 2, penultimate and antipenultimate joints of the flagellum subequal; flagellum 3 jointed. Gnathopod 1, second joint devoid of any spines on the upper margin; lower with a small spine at the distal end; fifth with 2 spines on the distal end on the upper margin and 14–16 slender spines on the inner margin. Gnathopod 2, with 7 small spines on the upper margin and 4 on the lower margin; fifth joint with 15–20 slender spines on the inner margin. Uropod 1, peduncle with 2 small spines on the lower margin and a row of 4 spines on the upper; outer ramus with 2 spines on the upper margin and the inner with 3; both the rami devoid of spines on the lower margins. Uropod 2, peduncle with a small spine at the distal end on the upper side. Both the rami with 2 spines

each on the upper margins. Uropod 3, with a single spine on the upper margin and a group of about 5 spines at the distal end. Length of the animal is about 4–5 mm.

Stage 6 (Fig. 6).—The head shorter than the first two segments combined. Antenna 1 with a single spine rather thin pointing forwards on the lower side of the first joint of the peduncle at the distal end; behind it are 2 stout spines; three more lateral spines on the lower side; second joint of the peduncle rather elongated, with about 10 setæ on the lower side and about 16 on the upper side; flagellum 15 jointed with sensory filaments on joints, tenth, twelfth, fourteenth and fifteenth. Antenna 2, peduncle much elongated; flagellum shorter than the last joint of the peduncle; flagellum 4 jointed; second and third joints with a curved spine at the middle of the



FIGS. 6–8. Growth stages of *Grandidierella bonnieri*. Fig. 6. Stage 6. Fig. 7. Stage 7. Fig. 8. Stage 8. (The gills and lamellæ are omitted from the drawings to avoid confusion of detail.)

segments. Gnathopod 1, fifth joint elongated; inner distal end rounded; lower margin with a large number of spines; sixth joint lower margin convex, a big spine at the middle and a smaller one behind. Gnathopod 2, fifth joint slightly convex at the lower side with a number of spines; sixth joint elongated, widening distally, both margins provided with

spines and setæ. Uropod 1, peduncle longer than rami; peduncle with 6 short spines on the upper margin and 3 on the lower; outer ramus and inner ramus each with 4 spines on the upper margins; both rami provided with the usual terminal spines. Uropod 2, smaller than uropod 1; outer ramus shorter than the inner; outer with 3 lateral spines on the upper side and 4 spines on the outer side in addition to the usual terminal spines. Uropod 3, peduncle short; rami with 2 spines at the upper margin and 5 spines at the distal end. Length of the animal is about 5–6 mm.

Stage 7 (Fig. 7).—Antenna 1, peduncle shorter than flagellum; first joint of the peduncle with 6–8 small setæ on the upper margin and with 2 or 3 slender spines at the anterior distal end; third joint of the peduncle about ⅓ the length of the second; flagellum 17 jointed with sensory filaments on tenth, twelfth, fourteenth, sixteenth and seventeenth joints. Antenna 2, third joint of the peduncle with a pair of stout spines on the upper margin and 2 or 3 lateral spines on the lower margin; penultimate and antipenultimate joints of the peduncle are subequal; flagellum 4 jointed, joints provided with setæ and curved spines. Gnathopod 1, fifth joint with a large number of spines on the lower side; 4 or 5 thin spines on the upper margin and about 3–4 at the anterior distal end; sixth joint with one stout spine at the middle on the lower margin and 3 in a row just behind it; seventh joint well developed and serrated in the inner margin. Gnathopod 2, fifth joint with a large number of slender spines on the lower margin and 3 or 4 on the upper margin; sixth joint with a row of 3 small spines on the lower margin and a slightly longer one at the posterior distal end. Peræopods 1 and 2 alike; sixth joint longer and tapering towards the distal end; seventh joint about as long as the sixth. Uropod 1, peduncle with 5 spines on the upper margin and 3 on the lower margin; outer ramus and inner ramus with 4 spines each on the upper margins. Uropod 2, like uropod 1, but much smaller. Uropod 3, ramus with 4–5 spines on the margin. Length of the animal is about 6–7 mm.

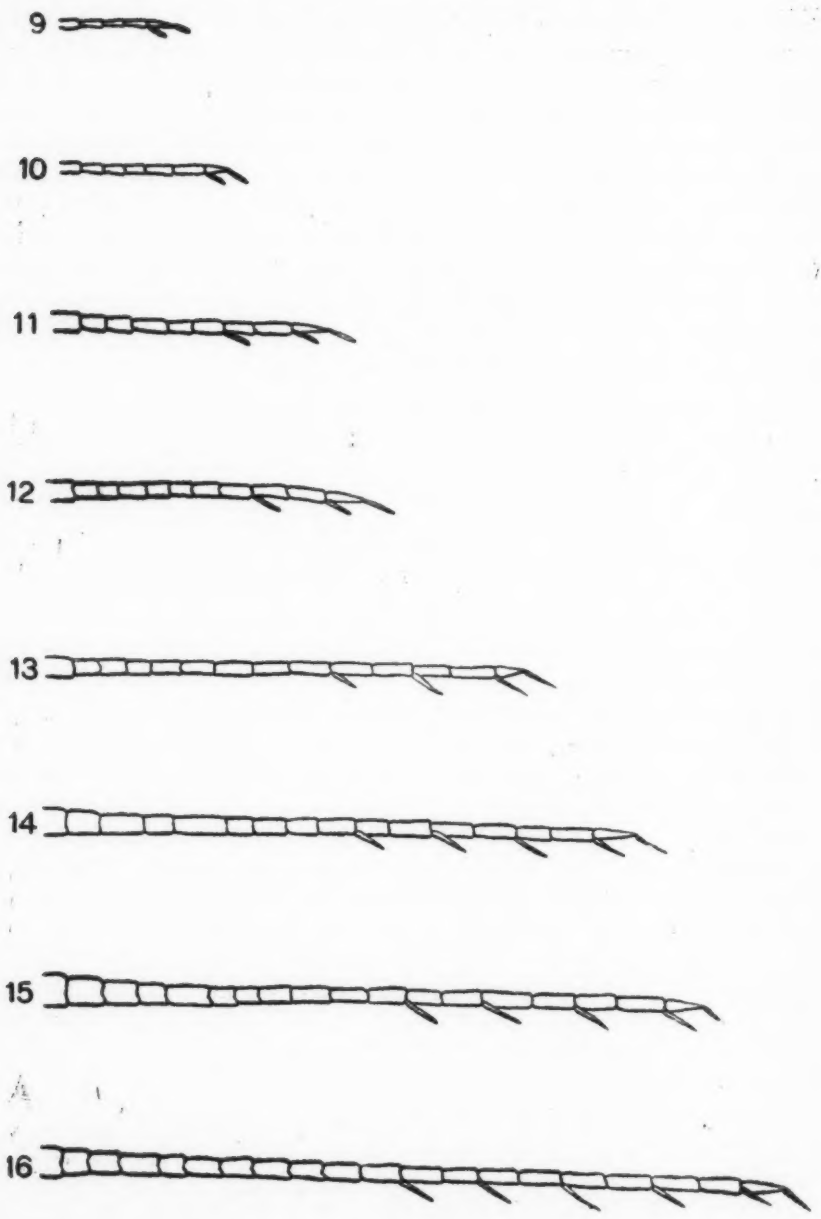
Stage 8 (Fig. 8).—Antenna 1, first joint of the peduncle more than half as long as second, lower side with 3 lateral spines and a small thin one at the distal end and 2 pairs of spines just behind it. Flagellum 19 jointed with sensory filaments on tenth, twelfth, fourteenth, sixteenth, eighteenth and nineteenth joints. Antenna 2, peduncle rather very well developed; flagellum subequal to the last joint of the peduncle; flagellum 5 jointed. Gnathopod, 1, fifth joint longer than the sixth; lower margin with a large number of spines; sixth joint with 4 stout blunt spines at the lower end; seventh joint well developed, curved. Gnathopod 2, fifth joint with a few spines on the

upper margin, lower margin with numerous long spines; sixth joint with 7 short lateral spines on the inner margin; palm differentiated. Peræopod 1 and 2 alike, fifth joint with 4 long and 3 short spines on the lower margin and a long and a short spine at the outer distal end and 3 short spines on the margin; sixth joint long and elongate. Uropod 1, peduncle with 6 lateral spines on the outer margin; outer ramus with 5 spines on the outer margin and 3 spines on the inner margin in addition to the usual terminal spines. Inner ramus with 2 spines on the outer margin and 5 spines on the inner margin. Uropod 2, rami unequal; outer ramus with 4 spines on the outer margin, inner ramus with 3 spines on the outer side and 5 on the inner side besides the usual terminal spines (4–5). Uropod 3, with 6 lateral spines, on the upper margin. Length of the animal is about 6–7 mm.

Differentiation of Features of Antenna 1 during the Growth

In antenna 1, the process of growth could be demonstrated better than in any other appendage. This process has been illustrated here with the aid of the drawings (Figs. 9–16), made from the successive moult stages from a female amphipod. The successive growth stages of the same animal were examined, throughout, to avoid the slight individual variation in the rate of development of different animals.

The young one, extruded from the brood pouch on 14th December 1948 had a peduncle which was 3 jointed and a short flagellum which was 4 jointed. The peduncle remained unchanged throughout its life, except for the fact that it increased in length and a few more spines and setæ were added on to it. The accessory flagellum was so small and was rather rudimentary with a few setæ at the tip of it. The first joint of the flagellum was devoid of any setæ at its distal end and the second joint with one spine on either side at its distal end. The third and fourth joints were provided with a sensory filament each at its lower side in addition to the 2 or 3 setæ present at the distal ends of the joints. At the tip of the fourth joint there appeared another very short joint, which was not counted, with 2 or 3 setæ at its tip. The first moulting took place on 17th December 1948 and the antenna 1 of the moult was examined and found identical with the description given above. In two days time (19th December) 2 more joints were added on to it and the sensory fllaments were seen on fifth, seventh and eighth joints (counting it from the base of the flagellum). In the fourth moulting stage (24th December), it was found to have the antenna 1 to be 10 jointed, but no more sensory filaments were added. This was followed by the fifth moulting stage (28th December) where the antenna 1 was found to have 13 joints. In this stage the sensory filaments were found on segments 8, 10, 12 and 13. It passed on



FIGS. 9–16. Differentiation of features of antenna 1 during the growth from birth to maturity. Only the sensory filaments are shown, the setæ being omitted.

to the sixth stage (5th January) and the antenna 1 in this stage was found to have 15 joints and had five sensory filaments on segments sixth, tenth, twelfth, fourteenth and fifteenth. In another six days time (11th January), it reached the seventh moulting stage. In this stage the antenna 1 was found to have 17 joints. It reached the last and the final stage in another 7 days time (18th January) wherein the antenna 1 was found to have 19 joints. The sensory filaments were found on segments tenth, twelfth, fourteenth, sixteenth, eighteenth and nineteenth. In all these cases the sensory filaments were present in the penultimate and antipenultimate joints. The preceding joint was devoid of a sensory filament and the one below had a sensory one. Thus it could be seen that the growth takes place not at the distal end but at the proximal end of the flagellum.

Summary

The life-history of a brackish water amphipod, *Grandidierella bonnieri* Stebbing, is described from the successive moult stages. The actual process of moulting is described. The changes that takes place in antenna 1, the gnathopods, the uropods and the peræopods, which have a very high taxonomic value, have been described in detail. The differentiation of features of antenna 1 during the growth has been illustrated and fully described.

Acknowledgements

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References

Barnard, K. H. .. "Report on some Amphipod, Isopoda, and Tanaidacea in the collection of the Indian Museum," *Res. Ind. Mus.*, 1935, **37**, 279–319.

Chilton, Charles .. "Fauna of the Chilka Lake," Amphipoda, *Mem. Ind. Mus.*, 1921, **5**, 521–58.

Sexton, E. W. .. "The Moulting and Growth stages of *Gammarus*, with Descriptions of the Normals and Intersexes of *G. chevreuxi*," *Journ. Mar. Biol. Assn.*, 1924, **13**, 340–401.

——— and Mathews, A. .. "Notes on the Life-history of *Gammarus chevreuxi*," *ibid.*, 1913, **9**, 546–56.

FURTHER STUDIES ON *PUCCINIA PENNISETI* ZIMM.

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In an earlier communication the complete life-history of the rust was described, following the discovery of its alternate host (Ramakrishnan and Soumini, 1948). Since then further studies were carried out and these have brought forth additional information on the alternate and collateral hosts of the rust, the prevalence of races and other features. The results of these studies are embodied in this communication.

After it was known that *Solanum melongena* L. served as the alternate host of this rust, field observations were made on the incidence of the acidial stage on brinjal in different tracts. It was noticed that on the Central Farm at Coimbatore, severe infection of brinjal occurred in fields adjacent to those where a heavily rusted crop of *Pennisetum typhoides* Stapf and Hubb. was present. At the Agricultural Research Station, Koilpatti, the brinjal crop was badly infected in 1954 and it was observed that a crop of *P. typhoides* severely infected by the rust was nearby. Heavy incidence of rust of brinjal was reported from Ramanathapuram District and here again the presence of the rust on *P. typhoides* in neighbouring fields was observed. Thus the infection of brinjal originating from cumbu (*P. typhoides*) was observed in nature also. A parallel development to what occurs between barberry and wheat is apparent here. But in this case both the hosts are of economic importance. Hence there is no question of eradicating the alternate host as a method of control of rust infection.

P. graminis Pers. is known to infect several species of *Berberis*. Experiments were conducted to find out whether *P. penniseti* could also infect more than one species of *Solanum*. Healthy plants of four species of *Solanum* and one variety of *S. melongena* besides the same species were raised in pots in the green-house and inoculated with germinating teliospores of *P. penniseti*. The inoculated plants were kept covered with alkathene bags for five days. The results obtained are shown in Table I.

It is evident from the results that the rust is able to infect at least four species of *Solanum*. *P. paspalicola* (Pat. and Gaill.) Arth. is also known to

* The subject-matter of this article formed part of the *Thesis* for the M.Sc. degree of the Madras University of Mr. N. V. Sundaram.

have *S. melongena* as an alternate host (Ocfemia, 1935). But the urediospores of *P. penniseti* do not infect *Paspalum scrobiculatum* L.

TABLE I

Results of inoculation on Solanum spp.

Name of the host	No. of leaves inoculated	No. of leaves infected	Incubation period in days for	
			Pycnia	Aecidia
S. melongena	10	10	7 to 10	10 to 15
S. melongena var. *insanum* Prain	10	8	12	15 to 16
S. pubescens Willd. ..	10	10	11	15
S. torvum Sw. ..	10	6	9 to 12	15
S. trilobatum L. ..	10	..	..	..
S. xanthocarpum Schrad & Wendl.	10	9	9	12

On all the species of *Solanum* hypertrophy of the infection spot occurs. Pycnia are formed on the upper surface and the æcidia mainly on the lower surface though they may sometimes develop on the upper surface and the petioles. The æcidia are often arranged in concentric rings. The pycnia and æcidia formed on the different hosts resembled one another and no differences could be made out in the dimensions of the spores.

The æciospores from the æcia on each of the species of *Solanum* were separately inoculated on the leaves of healthy plants of cumbu. In each instance uredia were developed in seven to ten days. No morphological difference could be made out between the uredia or the urediospores.

Pathogenicity of the rust on P. typhoides.—In nature the incidence of this rust is normally noticed just at the flowering stage though sometimes rust infection has been observed even from the seedling stage. There are instances when the crop has withered before flowering owing to heavy incidence of the rust. In order to find out whether the age of the crop has any influence on the intensity of infection of the rust, inoculations were conducted by spraying with an atomiser, suspensions of urediospores on the leaves of healthy plants of a susceptible variety, grown in the green-house, at intervals of 15 days from the time of sowing. The intensity of infection, two weeks

after inoculation estimated by using the modified Cobb's scale (Chester, 1946), is given in Table II.

TABLE II

Age of plants and intensity of infection

Age of plants in days	No. of leaves inoculated	No. of leaves infected	Intensity of infection
15	10	10	100
30	10	10	65
45	10	10	65
60	10	10	100
75	10	10	65
90	10	10	100

The rust is able to infect the host plant in all stages of growth provided humid conditions are maintained. The age of the plant had no influence on the intensity of infection in susceptible varieties.

Varietal reaction.—Numerous cultures were being cultivated at the Millets Breeding Station, Coimbatore. The reaction of agronomically important cultures among these was recorded for three successive seasons in the field. The intensity of infection is recorded in Table III.

Only three cultures exhibited low infection under field conditions. Of these the culture 814/3 had the lowest. In order to confirm the field observations healthy plants of the three cultures were raised in pots and subjected to artificial inoculation under green-house conditions. Only few sori developed on the leaves of 814/3 while on the others up to 40 per cent. infection was evident. The former culture behaved as a resistant one. But being a cross-fertilized crop its purity under field conditions will have to be maintained with all care if its cultivation spreads.

P. purpureum Schum., the elephant grass, was found to be immune to this rust. The Cytogeneticist, Coimbatore, had in his collection a number of hybrids of *P. typhoides* × *P. purpureum*, autotetraploids, amphidiploids, etc. These were being evolved with the object of producing rust-resistant and high-yielding strains to replace the susceptible cultivated varieties of

TABLE III

Intensity of infection (modified Cobb's scale) under field conditions

Culture	Intensity of infection (modified Cobb's scale)		
	1951–52	1952–53	1953–54
Co. 1	65	65	65
Co. 2	65	100	65
Co. 3	65	65	65
Co. 4	65	65	65
H. 865	40	40	40
H. 866	40	40	40
H. 874	25	25	25
H. 958	40	65	40
P.T. 814/3	..	Few sori	..
„ 816/2	40	25	40
„ 822/6	65	65	40
„ 825/4	65	65	65
„ 826/7	65	65	65
„ 826/4	100	65	100
„ 827/1	100	65	65
„ 827/2	100	65	65
„ 829/3	65	65	65
„ 829/5	5	25	25
„ 829/8	65	65	65
„ 833/3	65	65	65
„ 833/5	65	40	65
„ 834/4	65	65	65
„ 835/3	40	65	40
„ 835/6	65	40	65
„ 836/9	65	40	40
„ 865	65	65	65
„ 879	40	65	65
M.S. 6157/2–1	25	25	25
„ 6157/2–2	25	40	25
„ 6286	40	40	40
„ 6893	65	65	65
„ 6897	5	5	25

P. typhoides. Their reaction was assessed by artificial inoculation under green-house conditions. The results are recorded in Table IV.

Variation was evident in the intensity of infection among the different cultures. It has been reported that whenever the genomes of *P. typhoides* increased in the hybrids the susceptibility to the rust also increased (Ramakrishnan, 1952). Therefore the chances of producing a hybrid between *P. typhoides*

and *P. purpureum* which will combine resistance to the rust with high yield of grain are not bright.

TABLE IV

Results of inoculation of hybrids, etc.

Host	No. of leaves inoculated	No. of leaves infected	Intensity of infection
Amphidiploids ($2n = 42$)	10	9	25
Amphidiploids F_2 ($2n = 42$)	10	8	25
Autotetraploids			
20 B/1 ($2n = 28$)	10	9	40
24 A/4 ($2n = 28$)	10	10	40
13 A/1 ($2n = 28$)	10	10	25
4 N Cumbu III/21 ($2n = 21$) ..	10	6	5 to 25
P. typhoides × *P. purpureum*			
F_1 ($2n = 21$)	10	9	5 to 25
F_2 ($2n = 28$)	10	4	5
F_3 ($2n = 42$)	10	9	5
P. purpureum ($2n = 28$)	10	..	..
P. typhoides ($2n = 14$)	10	10	65

Collateral hosts.—This rust has been recorded on *P. leonis* from Africa. To determine whether other species of *Pennisetum* or allied grasses served as collateral hosts for this rust, inoculations were conducted on six species of *Pennisetum*, *viz.*, *P. alopecuros* Steud, *P. clandestinum* Hochst., *P. hohenackeri* Hochst., *P. orientale* Rich., *P. polystachion* Sch. and *P. rupellii* Steud. and two species of *Cenchrus*, *viz.*, *C. ciliaris* L. and *C. setigerus* Vahl. The rust infected *P. polystachyon* and *P. orientale* to a slight extent (about five per cent.).

Specialisation.—The large-scale cultivation of the alternate host and the diploidisation occurring prior to æcial formation are bound to result in the production of new races or physiological forms of the rust. This may give rise to new problems in the production of resistant host plants. A close study of the different types of infection spots formed on cumbu led us to suspect that more than one race may be present. The identification of the races of rusts is usually accomplished through the use of differential hosts if there are any. The cultivated varieties of cumbu showed two kinds of

uredia. In one these were isolated and scattered while in the other they were in groups on dark brown lesions. When urediospores from the former type were utilised for inoculation the resulting infection consisted of only isolated uredia. Inoculations carried out with the spores from the latter type similarly produced the corresponding type of infection only. Since all the cultivated varieties of cumbu exhibited both the types of sori other species and the hybrids (interspecific) of *Pennisetum* were inoculated. One hybrid of *P. typhoides* × *P. purpureum* was infected by the latter type alone while in the others both the types could be obtained. These inoculations were repeated several times and on every occasion the patchy type alone was able to infect this hybrid. Thus the existence of two races was established. Further studies may reveal the presence of more races.

Discussion

Puccinia penniseti has a wide distribution in India. In Madras State, the incidence of the rust on brinjal has been observed to be influenced by the presence of infected crops of cumbu in the vicinity. Similarly the spread of the rust from brinjal to cumbu is also possible. But it does not seem to be necessary that a brinjal crop is essential for the reccurrence of the rust on cumbu year after year. Just as in *P. graminis* this rust appears to be capable of surviving through the urediospores. Four species of *Solanum* serve as alternate hosts for this rust. Further search may reveal that more species are infected. Some unconnected species of *Aecidium* have been recorded on *Solanum*. It is possible that some of these belong to *P. penniseti*. *S. melongena* has also been known to be the alternate host of *P. paspalicola*, though it is not known whether other species of *Solanum* are also infected by this rust.

Two races of the rust have been recognised. More races may be discovered later. All the agronomically important varieties of cumbu cultivated in Madras State were found to be susceptible to the rust. The plant is found to be equally vulnerable in all stages of growth. The appearance of the rust in nature normally during or after the flowering stage is to be attributed only to the prevalence of favourable environmental conditions at that time.

One culture of cumbu has exhibited high resistance, though its agronomic value is not yet definitely known. Even if it is resistant now, it cannot be presumed that it will continue to behave so under cultivation as this is a cross-fertilized crop and is likely to lose its purity in a short period. Attempts are in progress to combine the resistance of *P. purpureum* with cumbu by hybridization. Here again there are indications that as the genomes of cumbu increase in the hybrid, the susceptibility to the rust also improves. Two collateral hosts for this rust have been observed and more may be noticed in

the future. The damage caused by this rust is being more and more recognised and more intensive work is called for to produce and multiply in isolated areas the resistant strains of this millet.

SUMMARY

Solanum melongena var. *insanum*, *S. pubescens*, *S. torvum* and *S. xanthocarpum* have been proved to serve as alternate hosts of *P. penniseti*. *Pennisetum polystachyon* and *P. orientale* were found to be collateral hosts. Two races of the rust have been differentiated. One culture of cumbu exhibited high resistance. Hybrids between *P. purpureum* and cumbu showed varying degrees of reaction towards the rust.

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REFERENCES

Chester, K. S. .. "Cereal rusts," *Chronica Botanica*, Waltham, 1946.

Ocfemia, G. O. .. *Philipp. Agric.*, 1935, **23**, 880–85.

Ramakrishnan, T. S. .. *Adm. Rep., Madras Agric. Dept. Rep. of the Govt. Mycologist for the year* 1951–52, 1952.

——— and Soumini, C. K. .. *Indian Phytopathology*, 1948, **1**, 97–103.

EXPLANATION OF PLATE

FIGS. 1–6. Fig. 1. Aecidia of *P. penniseti* on *S. melongena*. Fig. 2. Aecidia of *P. penniseti* on *S. pubescens*. Fig. 3. Aecidia of *P. penniseti* on *S. melongena* var. *insanum*. Fig. 4. Aecidia of *P. penniseti* on *S. torvum*. Fig. 5. Aecidia of *P. penniseti* on *S. xanthocarpum*. Fig. 6. Leaves of the hybrid of *P. typhoides* × *P. purpureum* showing infection by the race which forms sori in groups.

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FURTHER STUDIES ON THE RED HALOPHILIC BACTERIA FROM SOLAR SALTS AND SALTED FISH

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The 'red' brine organisms are of great economic interest as the causative agents of salted fish and hides. Though it is known that certain halophilic rods and cocci originating from solar salts are responsible for this, very few writers have given a systematic description of these bacteria (Harrison and Kennedy, 1922; Petter, 1932; Lochhead, 1934; Gibbons, 1936; Spruit and Pijper, 1952; Venkataraman and Sreenivasan, 1954; and Anderson, 1954). The inadequacy of the descriptions is inherent in the difficulty in the cultivation of these fastidious organisms in conventional media. Only Gibbons (1936) has given an excellent account of the media and methods suited for the study of red halophilic bacteria which was followed by us in our earlier work (1954). Recently Anderson (1954) has described the media used by him but these have no advantage over the classical media of Lochhead (1934) and of Gibbons (1936).

Methods of Study

Dussault and Lechance (1952) described an improved halophile medium which is a modification of Lochhead's salt-skim-milk-agar medium. The high demand of red halophiles for magnesium (Katznelson and Lochhead, 1952; Venkataraman and Sreenivasan, 1955) was also taken into account in devising this medium. With slight modification, in our hands, it has given exc lent results, giving a growth of red halophilic bacteria in 48–72 hours, or even less. Cultures of red halophilic bacteria were obtained from discrete colonies from plates used for enumerating these in various salts (Venkataraman and Sreenivasan, 1955). A few cultures were also obtained from salted fish having 'pink' attack. Morphological examination were made in hanging drops using 20% sterile brine. Gram staining was carried out as follows: Smears were prepared in 20% brine and fixed with methyl alcohol, or acetic-acid alcohol fixative. The stains used in gram's technique were added on to the methyl alcohol on slide. With this method the cocci appeared invariably gram negative or gram variable. But when smears were prepared in sea-

water and stained in the usual manner (excluding the above fixatives) all the cocci were gram positive. They were swollen very much but did not burst, whereas the rod forms plasmoptysed even when 10% brine was used for preparing smears. Flagella staining of motile cultures was done after fixation with methyl alcohol and staining by Leifson's technique. Though cell morphology was clear, flagella staining was unsuccessful. For studying the action on sugars, nitrate reduction, indole production, etc., liquid media used by some workers (Petter, 1932) were unsuitable. Even when enriched with yeast extract proteose peptone, neopeptone, tryptone, tryptose (Difco) or beef infusion, no growth was recorded in liquid media. For testing the action on sugars we used the Bacto Phenol-red agar base with 20% salt and the appropriate test sugar. For nitrate reduction and starch hydrolysis, salt-milk-agar was fortified with 0·5% KNO_3 and 0·2% starch respectively. Starch hydrolysis was also tested on Lochhead's medium A. Indole production was tested in a casitone yeast-salt broth with a filter-paper, partly dipping into the liquid, on which the red halophiles were grown. All these tests were made after 21 days and 1 month. Gelatin liquefaction was tested in the medium suggested by Gibbons (1936). Another medium was devised for recording the hydrolysis of gelatin. This contained proteose-peptone, yeast, salt and gelatin (0·5%) with 2% agar. Gelatin hydrolysis was tested by flooding the plates with 5% trichloracetic acid. H_2S was tested by flooding the salt-milk-agar plates or slants with heavy growth with lead acetate (Anderson, 1954).

DESCRIPTION OF THE ORGANISMS AND CLASSIFICATION

Rod forms.—The characteristics of the organisms isolated are summarized in Table I. The rod forms are divided into four groups. In group I are included five cultures which exhibit a very high degree of pleomorphism. These rarely, if any, grow in 10% salt concentration but show fairly good growth in 20% salt-milk-agar with a dirty orange-red colour. When, however, the salt concentration was increased to 25% the colour changes to deeper red. There were bizarre shapes and twisted forms reminding us of Pijper's (1952) description. Some of the cells showed the presence of 'vacuoles' which were also reported to be noted by Petter (1932) in *Bact. halobium*, Petter (*Bacillus halobius ruber*, Klebahn).

The nine cultures in group II are very minute rods, which show variations in form very rarely, if at all. They are actively motile but flagellation could not be determined. Growth on salt-milk-agar was very characteristic though slow and scanty. They showed slight growth in 72 hours and a maximum in 7–10 days, the colour being rose-red. It is adherent to the medium but is not rough or mucoid. Growth in other media did not occur.

Organisms in group III are bacillary forms, showing long filamentous, curved rods. Growth is rose to pink and highly mucoid, drawing out as fine thread on the needle. This group is physiologically more active than the others, digesting casein, very rapidly and liquefying gelatin. Growth was also noted in 10% salt but not on 3·5% salt. Good growth in 24–48 hours was obtained in 20% salt-milk-agar but in 25% it was slow and poorer. Organisms of group IV resembled *Halobacterium cutirubrum* (*Pseudomonas cutirubra*). This grew rapidly in 25% NaCl-milk-agar and exhibited beautiful variations in colour from pink, through scarlet red to mauve or coral red. This was a highly plemorphic culture if grown in 20% NaCl but simple bacillary forms were abundant in 25% NaCl. Though actively motile flagella could not be detected.

Coccus forms.—It is with the coccus forms that we experience difficulty in grouping. In the rod forms by mere appearance of cultures the four groups could be separated, which agreed with other physiological and morphological features too. Except the culture H 46 which was dirty-red to rose-red, all the others had a final deep red hue which did not differ materially from one another. On the basis of physiological properties we have grouped them into five divisions. The two cultures in group V appear to be *micrococcus*. They however differ from *Micrococcus morrhuæ* in the following respects—nitrates were not reduced, glucose, sucrose, and lactose turned alkaline and growth did not occur in 10% NaCl media.

Group VI comprised a single culture, H 46, which was a peculiar one. The original colony was 'wrinkled' with dirty rose-red colour. It was rapidly proteolytic in casein as well as in gelatin, and also amylolytic. It also produced indole. It is a distinctly new species. Group VII includes four strains. The three from salted fish are identical and resemble *Sarcina littoralis* to a large extent. But it differs from the latter in hydrolysing gelatin, producing alkali from glucose, and in regularly producing three-dimensional bundles in all media. The culture H 35 was also identical except that indole was produced. The culture H 30 in group VIII appeared morphologically similar to *S. littoralis* but differed markedly from it in many respects, for *e.g.*, gelatin was hydrolysed, nitrates were not reduced to nitrites. It also differed from *M. morrhuæ*.

The two cultures of group IX are also proteolytic in casein and gelatin but not as markedly in the former as in the latter. They differ from both *S. littoralis* and *M. morrhuæ* in many respects. The two former cultures turned sugars alkaline, did not reduce nitrates, and did not grow in 10% salt. Possibly they are new species of *Sarcina*.

TABLE I

Characteritics of the Red Halophilic Bacteria

Group	Strain No.	Morphology	Cultural characteristics	Growth in salt potato	Geltain		Casein digestion	Nitrate reduction	Action on sugars	Starch hydrolysis	Indol	H_2S	Growth in media with 10% NaCl	Source
					Liquefaction	Hydrolysis								
I	H. 17	Rods, pleomorphic, single and pairs, beaded clavate, cuneate, fusiform, spherical, and twisted rods. Bacillary, simple forms also. 0·9–2·0 × 1·2–2·5γ Non-motile; gram negative	Circular, entire, flat to slightly elevated smooth, glistening, beaded growth on salt-milk-agar. Rusty, orange-red, earlier but cinnabar red later, especially if NaCl is 25% or above; viscid, moist, growth	Rusty-red moist, glistening elevated streak turning dark later	Not liquefied	–	–	+	No change	–	–	–	–	Tuticorin sea salt
	18	Similar to H. 17 "Vacuoles" present	do	do	do	do	do	do	do	do	do	do	do	Adirampatnam salt
	19	Similar to H. 17	do	do	do	do	do	do	do	do	do	do	do	Ganjam salt
	33	Similar to H. 17 "Vacuoles" present	do	Orange red, streaks., scanty, glistening	do	do	do	do	do	do	do	do	do	Adirampatnam salt
	37	Similar to H. 17 "Vacuoles" present	do	do	do	do	do	–	do	do	do	do	do	Tuticorin salt

	37	Similar to H. 17 "Vacuoles" present	do	do	do	do	do	–	do	do	do	do	do	Tuticorin salt
II	H. 24	Rods, very small, almost coccoid, motile, simple rods. Flagella not possible to stain; gram negative, 0·5×0·7–0·8γ. Mostly single. Occasionally long bacillary forms also seen, but rarely pleomorphic	Rose red, circular, entire flat, coarsely, granular colonies, 2 mm. diameter. On salt-milk-agar slants, rose-red, effuse, thin, flat, adherent growth, slow growth	–	–	–	–	–	No change	–	–	–	do	Balacheruvu (Vizag) salt
	H. 26			do	do	do	do	do	do	do	do	do	do	Vajni (Wadala) salt
	27			do	do	do	do	do	do	do	do	do	do	do
	32			do	do	do	do	do	do	do	do	do	do	do
	34			do	do	do	do	do	do	do	do	do	do	Adirampatnam salt
	39			do	do	do	do	do	do	do	do	do	do	do
	41			do	do	do	do	do	do	do	do	do	do	Markanam salt
	42			do	do	do	do	do	do	do	do	do	do	Ganjam salt
	43			do	do	do	do	do	do	do	do	do	do	
III	H. 20	Rods, thin filamentous, curved, bent, single, pairs and shoft chains non-motile, gram negative. Rarely pleomorphic	Pale pink or rose, circular, elevated, entire, moist, glistening smooth 4–5 mm diameter. Moist, pink, filiform growth on slant	Scanty, brick red growth	Liquefied	Hydrolysed	++	–	Glucose–Alk (slow) Sucrose–Nc Lactose–Nc	+	–	–	+	Markanam salt
	21	As in H. 20. Spiral forms and beaded ones also noted	As in H. 20	do	do	do	++	–	As in H. 20	+	–	–	+	do
	45	Very long, filamentous, non-motile rods, thin, 0·6–0·8 ×2·5–2·0γ or more curved.	As in H. 20; Drawing out as fine thread on wire, mucoid	do	do	do	++	–	As in H. 20	+	–	–	+	Balacheruvu salt

TABLE I—(*Contd.*)

Group	Strain No.	Morphology	Cultural characteristic	Growth in salt potato	Gelatin		Casein digestion	Nitrate reduction	Action on sugars	Starch hydrolysis	Indol	H_2S	Growth in media with 10% NaCl	Source
					Liquefaction	Hydrolysis								
IV	H. 48	Rods, actively motile, pleomorphic, fusiform, clavate, spherical and bacillary. Mostly single	Rusty red to dark-red, circular, elevated, entire smooth, 2 mm. diameter; glistening. Old cultures become scarlet red Sometimes coral red to mauve also	–	Liquefied	Hydrolysed	Digested	–	No change	–	+ (slight)	–	–	Salted fish
V	23	Gram positive to variable large cocci 2·0–2·5γ, pairs and clusters, some in titrads	Circular, moist, brilliant red smooth, entire; on slant, abundant, filiform smooth	+	–	–	–	–	Glucose–Alk (slow) Sucrose–Alk Lactose–Alk	+	–	–	–	Didvana (Rajaputana salt)
	28			+	–	–	–	–		+	–	–	±	
VI	46	Cocci in packets of 16. 32 or more. 2·0γ gram negative or gram variable	Dirty-rose, circular, convex sligtly elevated "wrinkled" or folded; entire granular	–	Liquefied	+	++	+	Glucose–Alk Sucrose–Alk Lactose–Alk	+	+	–	+	Nagarcoil salt

VII	H. 14 15 16	Cocci, always in bundles of 16, 32, 64 or more in all media gram positive, large 2·5 γ diameter	Circular, raised, dark-red entire, smooth ; on slant, abundant; filiform, smooth red	–	–	+	–	+	Glucose–Alk Sucrose–Alk	–	–	–	–	Salted fish
	35	Cocci packets of 16 and above, gram negative	Vermillion red, circular, 1 mm. convex, smooth, entire	–	–	+	–	+	Lactose–Alk	–	+ (weak)	–	–	Kyarreshta salt (Sambhar lake)
VIII	30	Cocci, gram variable to gram positive packets of 4, 16, etc., also in pairs and short chains	Circular raised, 2–3 m.m, diameter, entire, smooth, glistening moist, dark-red	..	–	+	–	–	No change	..	+ (slight)	–	–	Balacheruvu salt
IX	36	Cocci in packets of 16 and above, also tetrads, 2·0γ gram possitive	Orange-red, circular, slightly elevated, confluent, folded, entire, smooth, glistening 2 mm. diameter	..	–	+	+ (slow)	–	No change	+	–	–	–	Vajni (Wadala) salt
	44	Gram positive, large cocci in packets of 4, 16 or above	Circular, bright red, convex, entire moist smooth	Rose red streaks	–	+	+	–	Glucose–Alk Sucrose–Alk Lactose–Alk	+	–	–	–	Balacheruvu salt

Alk — Alkaline; ± = Poor; .. — not tested.
* Gibbon's (1936) medium.

CLASSIFICATION

Rod forms of red salt bacteria have been variously classified under *Serratia*, *Pseudomonas* and *Bacterium* from time to time, with some workers (Klebahn) even naming them as *Bacillus*. Anderson (1954) has however proposed to include all these organisms under a new genus *Halobacter* in the family *Pseudomonadaceæ*. But Breed (1955) has proposed that these may be included in the new genus *Halobacterium* Elizari Volcani 1940 in the family *Pseudomonadaceæ*, since the latter has priority. He has rejected *Halobacterium* (Schoop, 1934) suggested by us because of incomplete description by Schoop. We agree with Breed's suggestion with regard to polar flagellated, red, obligately halophilic rod-shaped bacteria. In view of the fact however that flagella staining is difficult because of high salt concentration (Spruit and Pijper, 1952; Anderson, 1954) and in view of the fact non-motile forms also occur as reported herein the position has to be reviewed. We feel that all these obligatory halophilic rods, having red chromogens, may be placed in the genus *Halobacterium*. Spruit and Pijper (1952) and Anderson (1954) have also followed this. On the basis of this our cultures are named as follows:

Group I. *Halobacterium indicum* n. sp.

Group II. *Halobacterium minutum* n. sp.

Group III. *Halobacterium gibbonsii* n. sp.

Group IV. *Halobacterium cutirubrum* Lochhead.

As for the cocci we refrain from naming them for the present, except the one *viz.*, H 46 which is considered to be a distinctly new species. It is named *Sarcina marina* n. sp. Cultures of group VII also appear to be new species of *Sarcina*.

DISCUSSION AND CONCLUSIONS

Certain characteristics enumerated in Table I are noteworthy. It is seen that none of the rod forms produced acid from sugars conforming with Anderson's (1954) observations and those of Gibbons' (1936). Most of the cocci produced alkali from glucose, sucrose and lactose. Starch hydrolysis was marked by cultures of group III. Groups I and II were biochemically inert and hence their role in the spoilage of cured fish is questionable. However these two groups grew well and produced 'spoilage' odours and ammonia from Prawn-infusion-starch-salt medium. Not so the other cultures. Groups III and IV among the rods were strongly proteolytic. Likewise among the cocci group VI was strongly proteolytic, while groups IX and VII

were so to a lesser extent. Some of these also produced indole. Quite probably they may affect the quality of cured fish.

In view of the fact that flagella staining of halophiles is difficult and that non-motile forms also occur the importance of flagella in taxonomical differentiation is reduced. Hence the shifting of the red halophilic rods from the genus *Pseudomonas* to *Halobacterium* a new genus is welcome. It should include, all gram-negative, non-sporeforming red salt organisms which grow in salt concentrations of 10–30%, irrespective of whether they are polar flagellated or non-motile. The creation of this genus overcomes another incongruity, *viz.*, that the red salt bacteria do not possess either a soluble or greenish fluorescent phenazine pigment, which is characteristic of *Pseudomonas* (Tobie, 1945). Rod forms do not produce acid from sugars but according to Anderson (1954) utilise them. If the physiological pattern is shown to be oxidative, then relationship with *Pseudomonadaceæ* is established. The fact that most of these red halophilic bacteria fail to grow even in concentration of 10% NaCl shows that washing the curing plant and equipment with hot fresh water may be used to eliminate them (Castell, 1952).

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Literature Cited

1. Anderson, H. .. "The reddening of salted hides and fish," *Appl. Microbiol.*, 1954, **2**, 64.
2. Breed, Robert, S. .. (Personal communication), 1955.
3. Castell, C. H. .. "The survival of red halophiles in water and brines," *J. Fish. Res. Bd. Can.*, 1952, **9**, 377.
4. Dussault, H. P. and Lechance, R. A. "Improved medium for red halophilic bacteria from salt fish," *ibid.*, 1952, **9**, 157.
5. Gibbons, N. E. .. "Studies on salt fish. I. Bacteria associated with the reddening of salt fish," *J. Biol. Bd. Can.*, 1936, **3**, 70.
6. Harrison, F. C. and Kennedy, M. E. "The red discolouration of cured cod fish," *Trans. Roy. Soc. Can. Ser. III, Sect. II*, 1922, **16**, 101.
7. Katznelson, H. and Lochhead, A. G. "Growth factor requirements of halophilic bacteria," *J. Bact.*, 1952, **64**, 97.
8. Lochhead, A. G. .. "Bacteriological studies on the red discolouration of salted hides," *Canad. J. Res.*, 1934, **10**, 275.

9. Petter, H. F. M. .. "Over roode en andere Bacterien van gazouten Visch," *Thesis utretcht*. 1932.

10. Schoop, Gerhardt .. "Halophile Mikroben in Nahrungsmitteln," *Deutsch Tierärtl. Wochschr.*, 1934, **42**, 816.

11. Spruit, C. J. P. and Pijper, A. J. "An obligate halophilic bacterium from solar salt," *Antonie van Leeuwenhoek.*, 1952, **18**, 190.

12. Tobie, W. C. .. "A proposed biochemical basis for the genus *Pseudomonas*," *J. Bact.*, 1945, **49**, 459.

13. Venkataraman, R. and Sreenivasan, A. "Studies on the red halophilic bacteria from salted fish and salts," *Proc. Ind. Acad. Sci.*, 1954, **39**, 17.

14. ———— .. "The red halophilic bacterial content of various salts," *J. Sci. Ind. Res.*, 1955, **14 B**, 606.

STUDIES ON INDIAN ICHNEUMONIDAE (HYMENOPTERA)

Sub-family: BANCHINAE. Part III. Tribe BANCHINI

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SYSTEMATICALLY ARRANGED CATALOGUE OF INDIAN SPECIES

Tribe 3. BANCHINI

Banchini Ashmead, *Proc. U.S. nat. Mus.*, **23**: 96, (1900). Townes, *Mem. Amer. ent. Soc.*, **11** (1): 462, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric., Monograph*, **2**: 316, (1951).

Banchides Morley, *Ichn. Brit.*, **3**: 281, (1907); *Fauna Brit. India, Hymen.*, 3: 243, (1913).

Exetastes Gravenhorst

Exetastes Gravenhorst, *Ichn. Eur.*, **3**: 385, (1829), (Type of genus: *Ichneumon fornicator* Fabricius). Dalla Torre, *Cat. Hymen.*, **3**: 68, (1901–02). Morley, *Ichn. Brit.*, **3**: 289, (1907); *Fauna Brit. India, Hymen.*, **3**: 243, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (1): 462, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agri. Morograph*, **2**: 316, (1951).

[1]Range. Europe, Madeira, Teneriffe, Himalayas, Malay Archipelago, North America, Brazil.

Leptobatus Gravenhorst, *Ichn. Eur.*, **3**: 432.

Rhimphalea Förster, *Verh. Naturh. Ver. Preuss. Reinl.*, **25**: 202, (1868).

Alexetastes Kokujev, *Rev. Russ. Ent.*, **4**: 1 6, (1904).

? Tegona Morley, *Fauna Brit. India. Hymen.*, **3**: 251, (1913).

Pseudexetastes Meyer, Konowia, **6**: 308, (1927).

Tetractenion Seying, *Acad. Malagache Mem.*, **11**: 167, (1932).

cinctipes (Retzius).

Ichneumon spec Geoffray, *Hist. abr., Ins.*, **2**: 356, n. 57, (1762).

Ichneumon atrator Förster, *Nov. spp. Ins.*, p. 84, (1771).

Ichneumon cinctipes Retzius, *Gen. and spec. Ins.*, p. 168, n. 269, (1783). ♀. Villers, *C. linnæi Ent.*, **3**: 187, n. 162, (1789).

Ichneumon junci Foureroy, *Entom. Paris*, **2**: 413, n. 58, (1785).

Ichneumon osculatorius Fabricius, *Mant. Ins.*, **1**: 261, (1787); *Ent. Syst.*, **2**: 142, n. 38, (1793). ♂. Villers, C., *linnæi Ent.*, **3**: 151, n. 50, (1789). Gmelin, *Linne. Syst. Nat.*, Ed. 13*a*, **1** (5): 2680, n. 92, (1790). ♂. Oliviers, *Encycl. Meth. Ins.*, **7**: 170, n. 31, (1792). Walckenær, *Fauna Paris*, **2**: 53, n. 27, (1802). ♂. Tunberg, *Mem. Acad. Sci., St. Petersburg*, **9**: 302, (1824). ♀. Gravenhorst, *Ichn. Eur.*, **3**: 938, n. 90, (1829). Vallot, *Mem. Acad. Sc. Dijon*, p. 244, (1836).

Ichneumon clavator Fabricius, *Ent. Syst.*, **2**: 151, n. 74, (1793). ♀. Jurine, *Nouv. meth. class. Hymen.*, p. 107, (1807). ♂, ♀.

Cryptus osculatorius Fabricius, *Syst. Piez.*, p. 76, n. 23, (1824). ♂.

Ophion clavator Fabricius, *Syst. Piez.*, p. 134, n. 17, (1804). ♀. Panzer, *Krit. Revis.*, **2**: 89, (1806). Olivier, *Encycl. meth. Ins.*, **8**: 512, n. 23, (1811). ♀.

Ophion tarsator Fabricius, *Syst. Piez.*, p. 134, n. 20, (1804). ♀. Olivier, *Encycl. Meth. Ins.*, **8**: 512, n. 26, (1811). ♀.

Banchus clavator Gravenhorst, *Nov. acta. Acad. nat. curious*, **9**: 294, (1818). ♂.

Ichneumon tarsator Thunberg, *Bull. Acad. Sc. St. Petersburg*, **8**: 258, (1822) ♀; **9**: 254, (1924). ♂.

Exetastes osculatorius Gravenhorst, *Ichn. Eur.*, **3**: 413, n. 15, (1829). ♂. Parfitt, *Rep. and Trans. Deven. Assoc.*, **13**: 272, (1881).

Exetastes clavator Gravenhorst, *Ichn. Eur.*, **3**: 405, n. 11, (1829). ♀. Boie, *Stettin. Entom. Zeitg.*, **16**: 93, n. 23, (1935). Liehold, *Zeitschr. f. Ent.*, **4**: 388, (1843). Vollenhoven, *Pinacogr.*, pl. 17, fig. 3, ♂, ♀.

Tryphon tarsator, Zetterstedt, *Ins. Lapp.*, **1**: 336, n. 13, (1838). ♂, ♀.

Exetastes tarsator Holmgren, *Svenska vet. Akad. Handle*, n. 8, p. 158, (1858).

Exetastes cinctipes Thomson, *Opusc. ent.*, **22**: 2414, n. 1, (1899). ♂, ♀. Dalla Torre, *Cat. Hymen.*, **3**: 69, (1901–02). Morley, *Ichn. Brit.*, **3**: 304, (1907); *Fauna Brit. India*, **3**: 244, (1913). ♂, ♀.[1] [1]Europe, India.

fornicator Fabricius

Ichneumon fornicator Fabricius, *spec. Ins.*, **1**: 352, n. 72, (1781), ♀; *Mant. Ins.*, **1**: 267, n. 88, (1787); *Ent. Syst.*, **2**: 168, n. 144, (1793). Gmelin, *Linne, Syst. Nat.*, Ed. 13*a*, **1** (5): 2692, n. 150, (1790). Coderhjelm, *Fauna Ingric. Prodr.*, p. 161, n. 496, (1798). Jurine, *Nouv. meth. Class. Hymen.*, p. 107, (1807). ♀.

Banchus fornicator Fabricius, *Supply. entom. system.*, p. 233, n. 3, (1778); *Syst. Piez.*, p. 127, n. 3, (1804). Panzer, *Krit. Revis*, **2**: 86, (1806). Gravenhorst, *Nov. acta. Acad. nat. Curious*, **9**: 292, (1818). Trentepohl, *Isis.*, p. 964, n. 80, (1829). ♀. Blanchand, *Hist. nat. Insect.*, **3**: 322, 42, (1840). Dumeril, *Mem. acad. Sc. Paris*, **29**: 897, n. 3, (1860).

Exetastes fornicator Gravenhorst, *Ichn. Eur.*, **3**: 402, n. 10, (1829). ♂, ♀. Westwood, *Introd. Mod. classif. Insect.*, **2**: 60, (1840). *Boie, Stettin. entom. Zeitg.*, **16**: 92, n. 22, (1855). Holmgren, *Svenska vet. Akad. Handle*, n. 8, p. 150, (1858); *Ofv. Svensk. Vet. Akad. Porh.*, **15**: 329, n. 23, (1858). Vollenhoven, *Pinæcogr.*, pl. 17, fig. 2. Thomson, *Opusc. ent.*, **22**: 2415, n. 4, (1899). ♂, ♀. Dalla Torre, *Cat. Hymen.*, **3**: 71, (1901–02). Morley, *Ichn. Brit.*, **3**: 303, (1907); *Fauna Brit. India, Hymen.*, **3**: 250, (1913). ♂, ♀.[1] [1]Kashmir. Europe.

lucifer Morley

Exetastes lucifer Morley, *Fauna Brit. India, Hymen.*, **3**: 248, (1913). ♂.[1] [1]Assam.

nigripes Gravenhorst

Exetastes nigripes Gravenhorst, *Ichn. Eur.*, **3**: 416, n. 17, (1829). Excl. var. Ratzeburg, *Ichn. d. Forst. Inst.*, **3**: 94, (1852). Holmgren, *Svenska vet. Akad. Handl.*, p. 26, (1854); n. 8, p. 153, (1858). Thomson, *Opusc. ent.*, **22**: 2315, (1897). ♂, ♀. Morley, *Ichn. Brit.*, **3**: 296, (1907); *Fauna Brit. India, Hymen.*, **3**: 247, (1913). ♂, ♀.[1] [1]Sikkim: Darjeeling.

Var. *Exetastes illusor* Gravenhorst, *Ichn. Eur.*, **3**: 427, (1829). Holmgren, *Svenska. vet. Akad. Handle*, n. 8, p. 151, (1858). Thomson, *Opusc. ent.*, **22**: 2416, (1897). ♂, ♀. Morley, *Ent. Mo. Mag.*, p. 160, (1903). ♀; *Ichn. Brit.*, **3**: 297, (1907). ♂, ♀. *Fauna Brit. India, Hymen.*, **3**: 247, (1913). ♂, ♀.[1] *cf.*, Vollenhoven, *Pinac.*, pl. 17, fig. 4. [1]Europe, Sikkim: Darjeeling.

nitidus Cameron

Exetastes nitidus Cameron, *J. Bom. nat. Hist. Soc.*, **274**, (1906). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 249, (1913). ♀.[1] [1]Baluchistan: Quetta.

rufipes (Morley)

Tegona rufipes Cameron, MS. ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 251, (1913). ♂, ♀.[1] [1]Punjab: Simla.

Exetastes (= *Tegona*) *rufipes* Townes, *Mem. Amer. ent. Soc.*, **11** (1): 462, (1944).

tisiphone Morley

Exetastes tisiphone Morley, *Fauna Brit. India, Hymen.*, **3**: 246, (1913). ♂[1] [1]Sikkim.

Banchus Fabricius

Banchus Fabricius, *Sup. Ent. System.*, p. 209, (1798), (Type of genus: *Banchus pictus* Fabricius). Dalla Torre, *Cat. Hymen.*, **3**: 62, (1901–02). Morley, *Ichn. Brit.*, **3**: 281, (1907); *Fauna Brit. India, Hymen.*, **3**: 253, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11**: (1): 474, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dept. Agric. Monograph*, **2**: 320, (1951). [1]Range. India, Europe, Canada, United States, Brazil.

Corynephanus Wesmæl, *Soc. Roy. Belg. Bull.*, **16**: 631.

Cidaphurus Förster, *Verh. Naturh. Ver. Preuss. Reinl.*, **25**: 159, (1868).

armillatus Morley

Banchus armillatus Morley, *Fauna Brit. India, Hymen.*, **3**: 254, (1913). ♂,[1] [1]Nicobar Islands.

flavomaculatus (Cameron)

Cidaphurus flavomaculatus Cameron, *Zeits. Hym. Dip.*, p. 346, (1904). ♂.

Banchus flavomaculatus, Morley, *Fauna Brit. India, Hymen.*, **3**: 255, (1913). ♂.[1] [1]Punjab: Simla.

nox Morley

Banchus nox Morley, *Fauna Brit. India, Hymen.*, **3**: 255, (1913). ♂.[1] [1]Assam: Shillong, Khasi Hills.

Fintona Cameron

Fintona Cameron, *J. Bom. nat. Hist. Soc.*, p. 726, (1909), (Type of genus: *F. nigripalpis* Cameron). Morley, *Fauna Brit. India, Hymen.*, **3**: 257, (1913).

nigripalpis Cameron

Fintona nigripalpis Cameron, *J. Bom. Nat. Hist. Soc.*, p. 726, (1909). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 257, (1913). ♀.[1] [1]Punjab: Simla.

Eponites Cameron

Eponites Cameron, *Zeits. Hymen.-Dip.*, p. 77, (1905), (Type of genus: *E. ruficornis* Cameron). Morley, *Fauna Brit. India, Hymen.*, **3**: 258, (1913).[1] [1]Range, Northern India

ruficornis Cameron

Eponites ruficornis Cameron, *Zeits. Hymen.-Dip.*, p. 77, (1905) ♀; *J. Bom. nat. Hist. Soc.*, p. 291, (1906). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 259, (1913). ♂,[1] ♀. [1]Kashmir. Sikkim: Darjeeling; Bengal; Pusa.

scutellaris Morley

Eponites scutellaris Morley, *Fauna Brit. India, Hymen.*, **3**: 260, (1913). ♀[1]. [1]Bengal: Pusa.

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4

OBSERVATIONS ON THE WILT DISEASE OF THE BANANA

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The wilt disease of banana is prevalent in most of the countries where banana is grown, being caused by *Fusarium oxysporum* var. *cubense*. It affects several varieties and is known to be particularly severe on Gros Michel. The Cavendish group contains varieties known to be immune or highly resistant to wilt.

In India the disease has been gaining in importance in recent years. It was first reported from Chinsurah in 1911 (Basu, 1911). Since then reports of its occurrence in Bombay, Madras and Hyderabad are increasing. In Bombay the Kali and Basrai varieties are known to be resistant (Uppal, 1940). In Madras the varieties Monthan, Rasthali, Pachanadan and Tirunelveli Peyan suffer more from wilt, than Poovan. Park (in Wardlaw, 1935) has recorded that Mondan, Sonamondan, Kattumondan and Kolikkuttu varieties were found to be susceptible in Ceylon. Owing to the increased incidence of the disease in Madras State in recent years a detailed study of the malady was undertaken and the results obtained are described in this paper.

In the Madras State over a hundred varieties of bananas are under cultivation. Some varieties have a restricted region of distribution while others are more widely grown. Sundararaman (1928–29 to 1934–35) found that the disease was confined to a few varieties in each district. In Tiruchirapalli District, Rasthali was affected while in Madura, Chinnamondan and Vellai were involved. He has also reported that Monthan was infected in more than one district. Pachanadan, Kullan and Anaikomban did not become affected even on artificial inoculation. It was also reported that the pathogen from the wilted plants had been identified by Wollenweber to whom the cultures had been sent as *F. cubense* var. *inodoratum*. Recently the disease was found to be prevalent in other districts also. The variety Neypoovan was severely infected at the Agricultural Research Station, Anakapalle, in 1949. On the Lower Pulneys the incidence of wilt is increasing in the variety Sirumalai. In Tirunelveli the variety Peyan is being severely damaged year after year. Monthan and Pachanadan are being destroyed in large numbers in Coimbatore District. The disease is observed to be more in light

soils and garden land conditions than in the wet lands. The incidence of wilt is widespread in the State of Madras.

MATERIALS AND METHODS

Detailed investigations were carried out on the reaction of over twenty vaieties of bananas common in the State and the cultural characters of the isolates of the pathogen were studied. The banana plants were raised from suckers carefully selected from an area where wilt had not been noticed at any time. They were planted in big mud pots filled with garden soil. The pathogen was isolated from single spores and maintained on Brown's agar. In testing the reaction of the varieties the isolate from Monthan was used. The agar media were adjusted to pH 6 except where otherwise stated. The cultures were maintained at the laboratory temperature (26–31° C.). Measurements of spores were always made from cultures 21 days old.

EXPERIMENTAL RESULTS

In order to standardise the inoculation technique and select the most effective one for general adoption Monthan plants were inoculated in different ways. Cultures of the pathogen grown on sterilised bits of the rhizome or sand-oats medium were mixed with the soil in the pots after the plants had established; suspensions of the spores were poured into the soil at the base of the plants; the roots of the plants were exposed in four places and the culture of the fungus grown on oats agar was placed in contact with them and covered up with soil. Successful and consistent infection was obtained with the first two methods and these were adopted in all later infection experiments. The plants wilted completely in sixteen weeks.

Varietal Reaction.—Eighteen varieties were included in these trials to assess their susceptibility to this disease. The plants were inoculated after they had established in the pots and had begun to put forth fresh leaves. The results are given in Table I.

The control plants in all the varieties remained healthy and grew normally. The infected plants were invariably stunted and had thinner pseudostems. The wilting of the plants and the splitting of the outer sheaths were characteristic. On removal from the pots it was found that in many cases the roots were discoloured and had been destroyed to a large extent. When the rhizome was cut open the characteristic violet streaks and patches were visible. In all cases the pathogen was reisolated from the infected plants. Five varieties were not infected at all. Nor did they exhibit any stunting of the growth. These were Moongil, Poovan, Peyladen, Rajabale and Vamanakeli. In the field also the disease is very rare in the last variety.

TABLE I

Varietal Reaction

Variety	No. inoculated	No. infected	Incubation period	Remarks
			Weeks	
Chakkarakeli	5	3	16	Infected plants wilted, tissues of rhizome discoloured
Gros Michel ..	5	5	8	,,
Kali ..	5	5	12	,,
Kapur ..	5	5	12	,,
Kullan ..	4	2	14	Two wilted, others stunted.
Mindoli ..	5	2	16	,,
Monthan ..	5	5	16	All wilted
Moongil ..	5	Nil	..	All healthy
Neypoovan ..	5	5	9	All wilted
Pachanadan ..	5	5	12	,,
Peyan ..	5	5	12	,,
Peyladen ..	5	Nil	..	All healthy
Poovan ..	5	,,	..	,,
Rajabale ..	5	,,	..	,,
Rasthali ..	5	5	9	All wilted
Sirumalai ..	5	5	12	,,
Vamanakeli ..	5	Nil	..	All healthy
Vellaivazhai ..	5	5	16	All wilted

Vamanakeli belongs to the Cavendish group which is reputed to include several resistant and immune varieties. But Kullan which also belongs to the same group was partially infected. It is satisfying to note that there are a number of varieties which exhibit resistance to this disease. But attempts are being made to popularise the variety Gros Michel. A note of warning must be sounded here. This is a highly susceptible variety and its expansion will only result in the spread of this wilt.

In addition to the trials in the pots, infection experiments were conducted under field conditions, in wet lands. Healthy suckers of 28 varieties were planted in March after ploughing in a green manure crop of sunnhemp (*Crotalaria juncea*). The varieties were planted in two blocks, one for inoculation and the other to serve as control. After the plants had established the plants in one block were inoculated by mixing the contents of one 1000 c.c. flask of sand-oats culture of the pathogen with the soil, round each plant. At the end of sixteen weeks it was found that most of the plants excepting those of Poovan, Rajabale, Moongil, Peyladen and Vamanakeli

were much stunted and had thinner pseudostems. Young plants growing in inoculated soils have been observed to be severely stunted almost from the start in other countries also (Wardlaw, 1935). However all the inoculated plants of Kunnan, and Thattillakunnan wilted. In Karivazhai half the number of plants died while in Anaikomban, Ayiramkai Rasthali and Chenkadali a third of the plants wilted. The pathogen was reisolated from the affected plants in all cases. The control plants which were in a separate block were all robust and normal. The results also showed that infection does not spread rapidly under wet land conditions as in the pots. Therefore such conditions do not appear to be ideal for testing the susceptibility of the varieties.

The spread of infection from the mother plant to the suckers could be readily traced in many on cutting through the rhizome. There was significant difference between the root system of the infected plants and the controls. In the latter many long whitish roots could be seen while in the wilted plants the roots were few and most of them were dead and brown in colour. In a few all the roots had been destroyed.

The pathogen readily infects some varieties of banana but not others. In order to find out whether the resistance of some of the varieties is due to any inhibitory influence of the sap, the fungus was grown on media to which the sap of particular varieties had been added. Extracts of the rhizome and roots of Monthan, Gros Michel, Vellaivazhai, Poovan, Peyladen and Vamanakeli were prepared and filtered through Chamberland's filter. These were added to Brown's agar at the rate of 10 c.c. to every 100 c.c. of the medium. The pathogen grew well on all these media and no difference could be made out in the rate of growth of the fungus on Brown's agar and on those to which the extracts had been added. Hence it is concluded that the sap does not exert any inhibitory influence (at the concentration used). The resistance must be attributed to some other factor.

Host Range.—To determine whether any other local economic crop plants served as collateral host for the pathogen, inoculation experiments were conducted on plants allied to banana and others known to be parasitised by other species of *Fusarium*. These included *Canna indica*, *Curcuma longa*, *Maranta arundinacea*, *Zingiber officinale*, and three-weeks-old plants of *Allium cepa*, *Cajanus cajan*, *Capsicum annuum*, *Crotalaria juncea*, *Cyamopsis tetragonoloba*, *Gossypium arboreum*, *Lycopersicum esculentum* and *Nicotiana tabacum*. The inoculations were made by adding the culture of the fungus grown on sand-oats medium to the soil at the base of the plants. In no case was there any evidence of infection. The inoculated and the control

plants were alike healthy. The pathogen appears to have a restricted host range.

Studies of the Pathogen

Cultural Characters.—To begin with, the pathogen was isolated from diseased Monthan plants and the characters of this isolate were observed. It grew well on the common laboratory media filling the Petri-dishes (10 cm.) in 8 days. Some differences were however noticed in the nature of growth, pigmentation and sporulation on the different media and these are described in Table II.

Table II

Characters of the Isolate from Monthan

Particulars		Potato dextrose agar	Oats agar	Brown's agar	Cooked rice
Growth		Mycelium submerged	Luxuriant aerial	Little aerial	White, looss
Pigmentation of medium		Shell pink with patches of indigo blue	Purple vinaceous to claret red turning pompeian red	Not appreciably pigmented	Starting with hermosa pink turns to violet purple
Odour		No odour	No odour	No odour	Lilac odour
Sporulation		++	+++	+	+++
Chlamydospores		Single or in pairs, 5–6×5–6 μ	Single or paired 6–9×5–9 μ	Mostly on conidia, single or paired 7–12×5–9 μ	Very rare
Septation groups and size of conidia in μ	0–	5·5–12·5×2·5–3·5 6×3	5·5–12×2·5–3·5 8×3	5–11×3–3·5 10×3·5	5–12×2·5–3·5 8×3
	1–	5·5–14×2·5–4 9·5×3	8–12·5×2·5–3·5 11×3	8–15·5×3–5 12×4	7–20×2·5–3·5 12×3
	2–	8·5–18·5×3 13×3	15–28×3–4·5 20×4	15·5–31×3–5 24×4	10–20×3–5 13×3·5
	3–	22–43×3–5 30×3·5	25–45×3·5–4·5 31×4	18·5–50×4–5 33·5×4·5	..
	4–	28–47×4–5 36·5×4·5	28–43×3·5–4·5 33·5×4	31–43×4–5 36×4·5	..
	5–	28–43×4–5 37×4·5	28–46×4–5 33×4·5	31–53×4–5 43×4·5	..

[*Note.*—The intensity of sporulation is indicated by + mark, the increase being denoted by the increase in the number of marks. The measurements are shown in two lines in each septation group, the upper figures denoting the range and the lower ones the average.]

The pigmentation varied with the medium, no pigment being formed in Brown's agar. Maximum sporulation was obtained on oats agar and cooked rice. Lilac odour was conspicuous in the culture on cooked rice. On the other media no odour could be detected. Chlamydospores and sickle-shaped macroconidia were very rare on cooked rice.

Temperature and Growth of the Isolate.—The temperature relations of the isolate were determined by growing it under different constant temperatures. The choice of the range of temperature was limited by the available equipment. The diameter of the growths was measured at 24-hour intervals and are recorded in Table III. The inoculum was cut out with a sterilised cork-borer giving 8-mm. discs.

TABLE III

Temperature and Growth of the Monthan Isolate

No. of days	Diameter of the growth in millimetres at					
	5° C.	15° C.	30° C.	33° C.	35° C.	37° C.
1	8	8	14	8	8	8
2	8	14	27	12	8	8
3	8	20	42	18	8	8
4	8	28	54	26	8	8
5	8	32	69	30	8	8
6	8	38	82	36	8	8
7	8	44	Dish full	42	8	8

The maximum growth was at 30° C. among the temperatures included in the trials. The growth is slowed down considerably at 15° and 33° C. practically to the same extent. The dishes kept at 5°, 35° and 37° C. were taken out and left at the laboratory temperature (27°–29° C.). In 24 hours, growth was evident in the first two sets but not in the dishes exposed to 37° C., indicating thereby that the fungus had lost its viability by such long exposure to this temperature.

Other Cultural Characters.—The pathogen was able to tolerate a wide range of pH, *viz.*, from 3 to 7·5. The best growth was at pH 4 to 6. The capacity of the organism to utilise different sources of nitrogen was tested by adding peptone, asparagin, urea, potassium nitrate and sodium nitrate, to a basic medium lacking in nitrogen. When these substances were added in such quantities as to contain equivalent amounts of nitrogen no differences could be made out between them either in the rate of growth or in the intensity of sporulation (up to one gram of nitrogen per litre of the medium).

Meridith (1941) has reported that monthly applications of two ounces of sodium nitrate to the soil per plant was beneficial. The spores are stated to be inactivated by adding one part of sodium nitrate to five parts of soil culture. But in our studies it was found that there was no effect at the concentration used. However the addition of 0·5 and 1 per cent. of urea to the medium had an adverse effect on the growth and sporulation of the fungus. This substance was added to the normal oats agar and the growths were compared. At the 0·1 per cent. level there was no appreciable difference between the growths and sporulation on oats agar and the urea added one. But as the amount of urea increased to 0·5 and 1 per cent. levels the diameter of the growths was very much reduced and sporulation became scanty.

The isolate was grown in dishes kept in complete darkness, in dishes exposed to diffuse light during the day and darkness during the night and in dishes exposed to continuous light with the help of two 100 watt bulbs, to note the effect of light on growth. There was no difference between them either in the rate of growth or in the intensity of sporulation.

Enzyme Production.—The enzymes produced by the fungus were determined. The capacity of the organism to grow on certain media was taken as an index of the formation of particular enzymes in the mycelium of the fungus (Uppal and Kulkarni, 1937). A basic medium containing $MgSO_4$ 0·5 gm., K_2HPO_4 1·0 gm., KCl 1·5 gm., ferrous sulphate 0·1 gm., agar 20 gm. and distilled water 1,000 c.c. was prepared. To this were added different substances for testing particular enzymes.

Amidase.—This was determined by growing the fungus on the medium after adding 0·5 per cent. asparagin and 5 c.c. of 2 per cent. rosolic acid to a litre of the medium. The medium had a light yellow tint to start with. When the fungus grew the colour changed to bright red indicating that the asparagin was being acted upon by amidase liberating ammonia which was responsible for the reddening.

Diastase.—Two per cent. soluble starch was added to the basic medium. The fungus produced a good growth and when the dish was half covered it was flooded with a solution of iodine. The medium under the growth and a margin of half a centimetre round it remained white while the rest was coloured dark blue. This indicated that the starch in the unstained portions had been utilised by the fungus by the production of diastase.

Emulsin.—Amygdalin was added to the basic medium. There was good growth on this medium showing thereby that the fungus is able to utilise this substance.

Erepsin.—Finely divided casein (one per cent.) was added to the basic medium. The medium was cleared of the bits of casein as the fungus grew, from below the growth and to a small distance in advance of the growth due to the digestion of casein by the production of erepsin.

Lipase.—Litmus cream agar was used to test the presence of this enzyme. The medium under the fungal mat and to some distance all round it assumed a bright red colour indicating the production of acid. Lipase was being produced which acted upon the fat resulting in the formation of acid.

Oxidase.—This was determined by growing the fungus in potato dextrose agar to which 0·25 per cent. of tannic acid had been added (Bavendam's method, in Ramakrishnan, 1941). A deep brown halo developed round the growth indicating the utilisation of tannin by the production of oxidase.

Trypsin.—Egg albumen agar was used. The growth of the fungus was good and the medium underneath the fungus was cleared as the albumen had been utilised, with the formation of trypsin.

Survival of the Fungus in the Soil.—The pathogen survives in the soil. Wardlaw (1941) has recorded that in land subjected to inundation, wilt is negligible. Stover (1954) reports that in soils under one and a half to three feet of water the fungus survived for 25 to 80 days. Under one inch of water in the laboratory the fungus survived from 45 to 165 days. The survival was more at lower temperatures than at 23–24° C. The length of time for which the fungus remained viable in the soil under local conditions was determined. Bits of the rhizome, infected with the fungus, were buried in the soil six inches below the surface in pots under different treatments. In one series the soil was thoroughly mixed with farmyard manure added at the rate of 5 tons per acre. In a second series lime was added to the soil at the rate of one ton per acre. In a third set of pots the soil moisture was maintained at 20 per cent. of its water-holding capacity. The soil moisture was maintained at 100 per cent. of its water-holding capacity in a fourth set and water was allowed to stagnate to a depth of two inches in the fifth set. At intervals of a fortnight isolations were made from the buried bits. The inability to isolate the pathogen was taken as a sign of the loss of viability of the fungus. It was found that the fungus could be isolated from the fifth series of pots (with two inches of stagnant water above the soil level) only for two fortnights after the treatment. In the limed soil the fungus retained its viability for four fortnights. From the other pots it could be isolated even after four months (after which the experiment was discontinued). This indicates that flooding the soil and application of lime to the soil will be useful in eradicating the fungus from the soil. The low incide ce of wilt

under wetland conditions may be due to the inability of the fungus to survive under those conditions in the soil.

Strains of the Pathogen.—During the course of these studies isolations were made from other naturally infected varieties of bananas. Two of these isolates, one from Pachanadan from Coimbatore and another from Kali from Lower Pulneys, were compared with the original isolate from Monthan. All of them were pathogenic to Monthan. But the two former isolates exhibited differences in other characters.

On potato dextrose agar the Monthan strain produced submerged growth while the other two gave rise to woolly aerial growths. On the different media, there were differences between the three in the depth of colour imparted to the medium. No odour was developed on cooked rice unlike in the Monthan isolate. The two new strains produced three to four or three to seven septate macroconidia respectively on cooked rice. Differences were evident in the size of the spores also. Thus more than one strain of the pathogen is present in the country. The pathogenic range of these strains and their distribution have to be taken into consideration before deciding on the suitability of different varieties of bananas to different tracts with reference to the wilt problem.

Summary

A detailed study of the wilt of banana was made. The assessment of the reaction of over twenty common varieties of the host showed that Poovan, Moongil, Peyladen, Rajabale and Vamanakeli were not infected when artificially inoculated. Infection studies conducted under wet-land conditions do not always give a correct picture of the reaction of the varieties but plants grown in big pots react satisfactorily. Susceptible varieties growing in infected soil are stunted in growth even though they may not always be killed. The pathogen does not infect any allied host-plants included in the studies. The resistant varieties do not have any pathogen-inhibiting substance in the sap.

The cultural characters of the fungus are described. The pathogen thrives best at about 30° C. and can tolerate a wide range of pH in the medium. The enzymes produced by the fungus have been determined. The pathogen does not survive in the soil for 45 days when two inches of water are maintained over it. When lime is added to the soil the fungus is inactivated in about two months. Under other conditions it remains viable for over four months. Strains of the pathogen isolated from Pachanadan and Kali exhibited differences in various characters from the isolate from Monthan.

We wish to express our gratitude to the Horticulturist and Professor of Horticulture, Agricultural College, Coimbatore, for arranging the supply of the suckers of different varieties of bananas and to the Superintendent, Central Farm, Coimbatore, for all the help in conducting the field trials.

LITERATURE CITED

Basu, S. K. .. *Quart. J. Dept. Agric. Bengal*, 1911, **4**. (4).

Meridith, C. H. .. *Phytopath.*, 1941, **31**, 564.

Ramakrishnan, T. S. .. *Proc. Indian Acad. Sci.*, 1941, **14B**, 395–411.

Stover, R. H. .. *Soil Sci.*, 1954, **77**, 401–14.

Sundararaman, S. .. *Adm. Rep. Govt. Mycologist, Dept. Agric., Madras*, for the years 1928–29 to 1934–35.

Uppal, B. N. .. *Rep. Dept. Agric., Bombay, Mycol. Work*, for the year 1938–39, 1940, 203–21.

——— and Kulkarni, N. T. *Indian J. Agric. Sci.*, 1937, **7**, 413–41.

Wardlaw, C. W. .. *Diseases of the Banana*, 1935, Macmillan & Co., London.

——— .. *Nature*, 1941, **147**, 380–81.

EXPLANATION OF PLATES

PLATE VIII

FIG. 1. Wilted Pachanadan in the field.

FIG. 2. Wilted Monthan showing splitting of sheaths.

FIG. 3. Culture of the pathogen in starch agar to which iodine solution was added to show the complete utilisation of starch under the fungus mat.

FIG. 4. Peyan infected; to the left is the control.

FIG. 5. Rajabale not infected; control is in the left extreme.

FIG. 6. Sirumalai infected, control to the left.

FIG. 7. Vamanakeli not infected; control left extreme.

FIG. 8. Vellaivazhai infected plants stunted; control left extreme.

FIG. 9. Kullan infected; control left.

FIG. 10. Kali infected; control left extreme.

FIG. 11. Chakkarakeli infected; control left.

PLATE IX

FIGS. 12, 13, and 17. Monthan, Chakkarakeli and Kullan respectively, showing the reduction and damage to the roots in the diseased plants; control left and diseased to the right.

FIG. 14. Infection spreading to the sucker from the parent rhizome.

FIG. 15. Mindoli infected; control left.

FIG. 16. Pachanadan infected; control left.

FIG. 18. Rasthali infected; control left.

FIG. 19. Kapur infected; control left.

FIG. 20. Neypoovan infected; control left.

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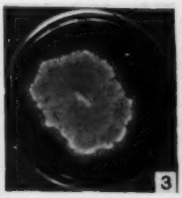
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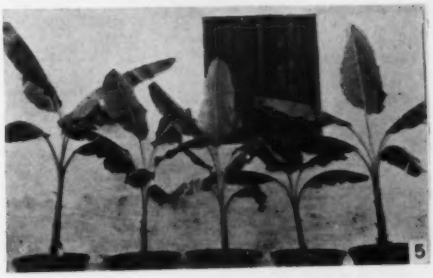
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THE RATE OF WATER FILTRATION IN THE MARINE WOOD-BORING MOLLUSC, *MARTESIA STRIATA* LINN.

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(Communicated by Prof. P. N. Ganapati, F.A.Sc.)

Introduction

In recent years studies on the rate of filtration in lamellibranchs have been made chiefly by Galtsoff (1926, 1928); Nelson (1936); Fox, Sverdrup and Cunningham (1937); Loosanoff and Engle (1947) and Jørgensen (1949). The techniques suggested by these workers are rather complicated requiring elaborate apparatus and careful preparation. The method recently used by Jørgensen (1949) relies upon mussels secreting mucus, as observed by MacGinitie (1941). Very recently Cole and Hepper (1954) described a simple technique using Neutral Red solution in sea-water which they employed for studying the variations in filtration rates in *Paphia*, *Ostrea edulis*, *Gryphæa* and *Cardium edule*.

The present work was undertaken with a view to study the rate of water filtration in *Martesia striata* using Neutral Red technique since no work seems to have been done on this form in this direction.

Material and Methods

Martesia striata used in these experiments were collected from standard test boards of dealwood, 1″ in thickness, 6″ in width and 10″ in length. The animals were removed carefully by cutting away the wood and placed in fresh sea-water in the laboratory before they are transferred into the experimental dishes.

Martesia striata are placed in a cylinder containing 100 ml. of 0·001% solution of Neutral Red in sea-water. Five animals of medium size, 15 mm., are taken usually in each experiment. To provide a standard, 100 ml. of the original solution (0·001%) is placed in a second cylinder. The two cylinders are held side by side over an evenly illuminated white background, shielded from direct light. At intervals of one hour, Neutral Red solution from the standard is removed with a pipette to a graduated measuring jar until, when viewed from above, the colour of the solutions in the cylinders

match. The amount of liquid (in ml.) left in the standard equals the percentage of Neutral Red not removed by *Martesia* and hence, by subtracting from 100, gives the percentage removed. To make the comparison easier, the sea-water is first filtered. All experiments are set up in uniform vessels on a firm bench not subjected to variations.

RESULTS

1. *General observations.*—The experiments recorded here were made in 1955. In each experiment five animals of uniform size were used. The salinity was 30·5‰. The percentage of Neutral Red removed was estimated

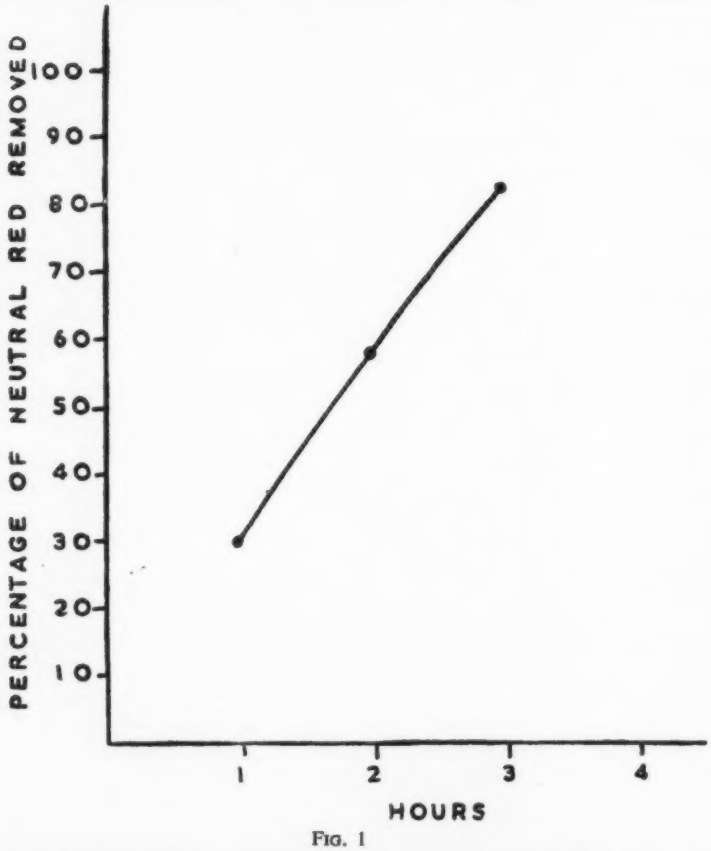

FIG. 1

at 1, 2 and 3 hours after commencement of experiment. Water temperature was recorded at the beginning and at the end of the experiment: it did not vary by more than 0·5° C. during the course of an experiment (3 hours).

The mean percentage of Neutral Red removed was calculated for five batches and the results are plotted in Fig. 1.

2. *Effect of low salinity on rate of filtration in* Martesia.—Batches of four *Martesia* of uniform size were used, each batch being placed in 100 ml. of water. The requisite range of salinity was produced by adding varying amounts of distilled water to sea-water. In salinities of 16·8‰ and 11·85‰, the animals are first acclimatized by placing them in these grades for a few hours. As soon as *Martesia* opened the shell valves, records of their filtration rates are made. Experiments were allowed to continue for 3–7 hours. A typical result is shown in Fig. 2. It appears that at low salinities *Martesia* filters at a slower rate than at normal salinity. In a previous paper of the author (1955) on the effect of low salinity on *Martesia*, it was mentioned that

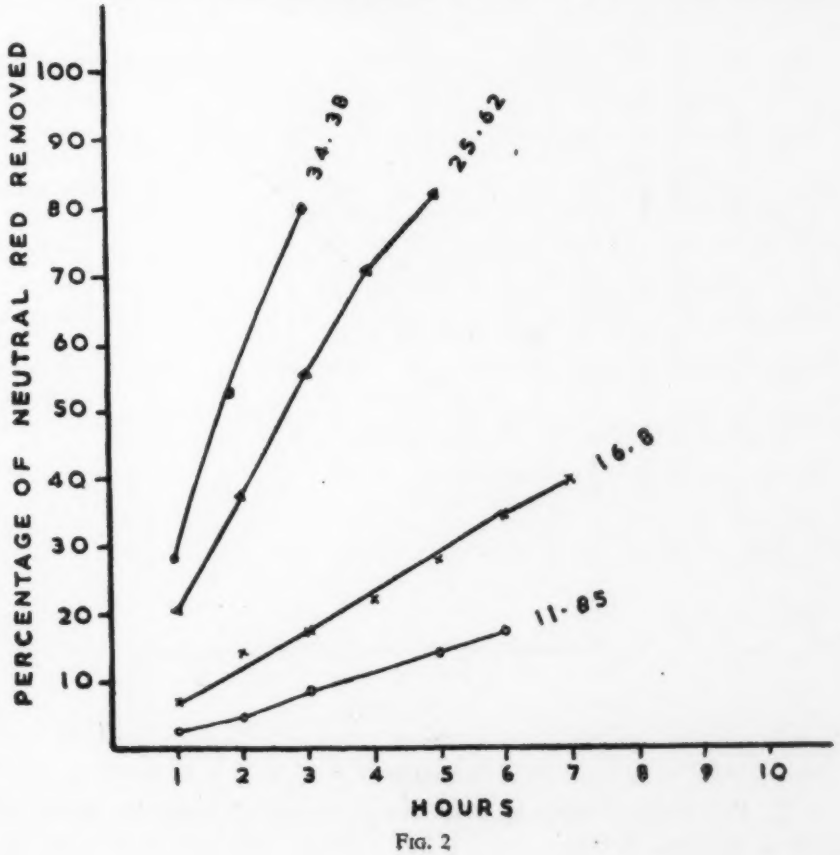

FIG. 2

there was progressive decrease in the amount of fæces discharged with decrease in salinity. The present results on the filtration rates in *Martesia* at low salinities confirm the above findings.

3. *Effect of light on filtration rate in* Martesia.—In order to see whether light has got any effect on the rate of filtration, two sets of experiments were conducted, using four *Martesia* in each. One set was placed in dark and the other in lighted area and the percentage of Neutral Red removed was estimated at hourly intervals. The results are plotted in Fig. 3. There appears to be no effect of light on water filtration rate in *Martesia.*

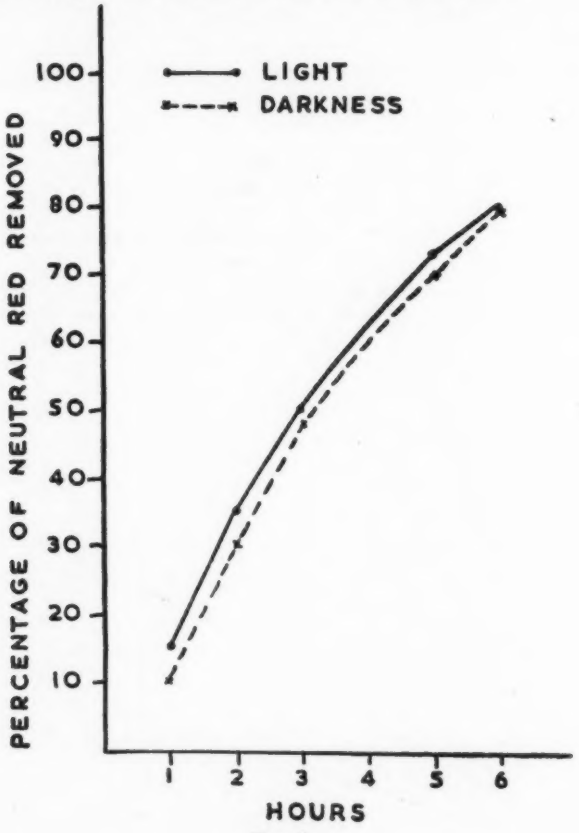

FIG. 3

SUMMARY

1. The Neutral Red technique was employed to study the filtration rates of *Martesia striata.*

2. It appears that low salinity has a great depressing effect upon the rate of filtration in *Martesia.*

3. Light has got no effect on the filtration rate.

ACKNOWLEDGEMENTS

I wish to express my grateful thanks to Prof. P. N. Ganapati, Head of the Department of Zoology, Andhra University, Waltair, for suggesting his topic for investigation and for guidance throughout the course of this work. This work was carried out with the funds provided by the Forest Research Institute, Dehra Dun, specially obtained from various sources for the execution of the scheme on the protection of timber against marine wood-boring organisms attack.

REFERENCES

Cole, H. A. and Hepper, B. T. "The use of Neutral Red solution for the comparative study of filtration rates of Lamellibranchs," *Jour. Du Cons.*, 1954, **20**, 197–203.

Fox, D. L. *et al.* .. "The rate of water propulsion by the California mussel," *Biol. Bull.*, 1937, **72**, 417–38.

Galtsoff, P. S. .. "New methods to measure the rate of flow produced by gills of oysters and other molluscs," *Science*, 1926, **63**, 233–34.

———— .. "Experimental study of the function of the oyster gills and its bearing on the problems of oyster culture and sanitary control of the oyster industry," *Bull. Bur. Fish.*, 1928, **44**, 1–39.

Jørgensen, C. B. .. "The rate of feeding of *Mytilus* in different kinds of suspensions," *J.M.B.A.*, U.K., 1949, **28**, 334–44.

Loosanoff, V. L. and Engle, J. B. .. "Feeding of oysters in relation to density of micro-organisms," *Science*, 1947, 105.

MacGinitie, G. E. .. "On the method of feeding of four pelecypods," *Biol. Bull.*, 1941, **80**, 18-25.

Nagabhushanam, R. .. "Tolerance of the marine wood-borer, *Martesia striata*, to waters of low salinity," *Jour Zool. Soc. India*, 1955, **7** (1), 83–86.

Nelson, T. C. .. "Water filtration by oyster and a new harmone effect upon the rate of flow," *Proc. Soc. Exp. Biol. and Med.*, 1936, **34**, 189–90.

THE NUCLEUS IN THE LIVING YEAST

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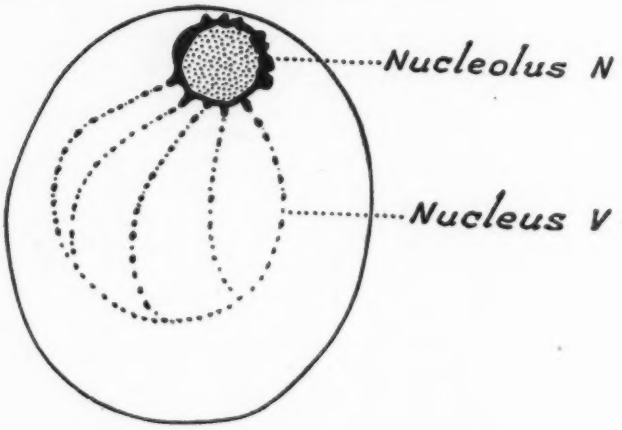
Introduction

In 1910 Wager and Peniston identified the vacuole in the yeast cell as its nucleus and a homogeneous body lying in the cytoplasm but in close contact with the vacuole as the nucleolus (Text-Fig. 1). During cell division the 'nucleolus' is said to constrict into two equal or unequal parts, one of which together with a part of the vacuole passes into the bud.



Text-Fig. 1. After Wager and Peniston, 1910.

The identification of these cell organelles has been in dispute and the following table would give an idea of the interpretations of the various investigators.

There appears to be a large measure of agreement regarding the identification of the homogeneous body as the nucleus. The differences relate only to the nuclear nature of the vacuole (1, 2 and 6 in table).

According to Wager and Peniston (1910) the 'nuclear vacuole' disappears during sporulation and the spore nuclei are said to originate by the division of the 'nucleolus' (p. 75). Nagel (1946) could not obtain any evidence that the vacuole takes part in meiotic phenomena. The "parvicorp" on the other hand was observed to exhibit changes suggestive of meiosis,

	Homogeneous Body	Vacuole
1. Janssens and Leblanc, 1898	Part of nucleus	Part of Nucleus
2. Wager and Peniston, 1910	Nucleolus ..	Nucleus
3. Guilliermond, 1920	Nucleus ..	Vacuole
4. Winge and collaborators, 1954 ..	Nucleus ..	Vacuole
5. Badian, 1937	Nucleus ..	Vacuole
6. Lindegren, 1952	Centrosome ..	Nuclear Vacuole
7. Nagel, 1946	Parvicorp ..	Magnicorp
8. Subramaniam and collaborators, 1946..	Nucleus ..	Vacuole
9. DeLamater and collaborators, 1950 ..	Nucleus ..	Vacuole
10. Lietz, 1951	Nucleus ..	Vacuole
11. Mundkur, 1954	Nucleus ..	V acuole

Lindegren (1945) revived the old concept of Wager and Peniston (1910) that the vacuole is the nucleus of the yeast cell, but in deference to modern ideas regarding the origin and location of the nucleolus, identified the ' nucleolus ' of Wager and Peniston as the ' centrosome '. During sporulation he observed an " apparent disappearance of the vacuole " and experienced difficulty in distinguishing the 'chromosomes' from the 'centrochromatin '. Widra and DeLamater (1955) conclude that the vacuolar contents are ergastic in nature.

To reconcile these divergences one has to locate the organelles under dispute in living cells. Wager and Peniston (1910) remark that the ' nucleolus ' is generally invisible in living cells but that occasionally " it can be distinguished as a slightly refringent body in contact with the central vacuole " (p. 49). Lindegren and Rafalko (1950) also report that only occasionally could the ' centrosome ' be observed as an " opaque area " in the cytoplasm.

Material and Methods

On the belief that a rational approach to a study of the staining reactions and behaviour of these cell organelles would be in a strain where these two structures could be observed together in unstained living cells, an extensive

survey of the yeast cultures in our collection was carried out. The growth medium employed was barley malt wort of S.G. 1·020 and pH 4·6–4·8. Cells from cultures of varying ages were examined first under a Zeiss Phase Contrast Research Microscope and later with a Bausch and Lomb microscope under ordinary as well as dark ground illumination. The photographs were taken with a Leica attachment on Kodak 35 mm. 'Microfile' film. The negatives were enlarged to varying degrees.

Observations

In cells of a 120-hr. culture of a strain of *Saccharomyces cerevisiæ*,—obtained through the kindness of Dr. Wickerham,—the two structures described by Wager and Peniston (*N*, *V*, Text-Fig.) could easily be observed. Photos 1 and 2 show their appearance under phase contrast. The vacuole (*V*) appears free of inclusions, but bordering it are grains of different sizes. The corpuscle (*N*) associated with the vacuole (Photos 1 and 2) is generally globular. In Photo 1 it lies partly over the vacuole while in Photo 2 it is merely in contact with it.

Photography with a light microscope is relatively difficult (Photo 3). Granules could be seen arranged irregularly along the boundary of the vacuole. The picture seen under dark ground illumination is superposable on those of phase contrast and light microscopes. According to Wager and Peniston (1910) the vacuole is not delimited from the cytoplasm by a membrane. Examination under dark ground illumination suggests the existence of a membrane surrounding not only the vacuole (*VM*, Photos 5 and 6) but also the body associated with it (*NM*, Photos 4 and 5).

Cell division was rather rare in the 120-hr. culture and the appearance of a budding cell under dark ground illumination is illustrated in Photo 6. The bud is free of any inclusions. The mother cell has a huge vacuole and near the bud is the nucleus (*N*). There appears to be a membrane (*M*) between the vacuole and the nucleus.

Discussion

Wager and Peniston observe: "The large size of the nuclear vacuole (Figs. in Pl. VI) is so disproportionate to the size of the cell and contains such a large quantity of clear sap in proportion to the very diffuse nuclear network, that failure to recognize it as a nucleus is not perhaps astonishing. But the extraordinary activity of the yeast cell at various stages in development explains the unusual size of the nuclear vacuole, and in the light of the large vacuolar nuclei associated with gland or other cells which exhibit very active metabolism, affords additional proof of its nuclear nature" (pp. 55–56)

(*cf.*, Royan and Subramaniam, 1954). This suggestion is further elaborated by them in the concluding part of the paper. Since the vacuole plays no part in sporulation they conclude that the nuclear vacuole is concerned purely with cell metabolism acting as a store-house for the accumulation and elaboration of chromatin. If their interpretation is accepted, then, on analogy with the condition in Ciliates the ' nucleolus ' (=' centrosome ' of Lindegren) would correspond to the germinal micronucleus and the vacuole to the vegetative macronucleus.

Cytological analysis has all along been on the assumption that yeast is uni-nucleate (Subramaniam and Royan, 1954). Can it be that yeast is bi-nucleate ?

Summary

1. There appears to be a large measure of agreement regarding the identification of the homogeneous body in the yeast cell as the nucleus. The differences relate only to the nuclear nature of the vacuole.

2. In a 120-hr. culture of a strain of *S. cerevisiæ*, the appearance of the nucleus and vacuole under ordinary, phase contrast and dark ground illumination is described. Each appears to possess a membrane delimiting it from the cytoplasm. Can it be that yeast is bi-nucleate ?

References

1. Badian, J. .. *Acad. Polon, Bull.*, 1937, **1B**, 61–87.
2. DeLamater, E. D. .. *Jour. Bact.*, 1950, **60**, 321–32.
3. Guilliermond, A. .. *The Yeasts*, Trans., F. W. Tanner, 1920.
4. Janssens, F. A. and Leblanc, A. *Cellule*, 1898, **14**, 201–43.
5. Liętz, K. .. *Arch. Mikrobiol.*, 1951, **16**, 275–302.
6. Lindegren, C. C. .. *Symp. Soc. Expl. Biol.*, 1952, **6**, 277–89.
7. — and Rafalko, M. M. *Expl. Cell. Res.*, 1950, **1**, 169–87.
8. Mundkur, B. D. .. *Jour. Bact.*, 1954, **68**, 514–29.
9. Nagel, L. .. *Ann. Mo. Bot. Gdn.*, 1946, **33**, 249–73.
10. Royan, S. and Subramaniam, M. K. *Curr. Sci.*, 1954, **23**, 315–19.
11. Subramaniam, M. K. .. *Proc. Natl. Inst. Sci., India*, 1946, **12**, 143–49.
12. — and Royan, S. .. *Curr. Sci.*, 1954, **23**, 351–54.
13. Wager, H., and Peniston, A. *Ann. Bot.*, 1910, **24**, 45-84.

14. Widra, A. and DeLamater, E. D. — *Am. J. Bot.*, 1955, **42**, 423–35.

15. Winge, O. and Roberts, C. — *C.R. Lab. Carlsberg. Ser. Physiol.*, 1954, **25**, 285–327.

EXPLANATION OF PLATE

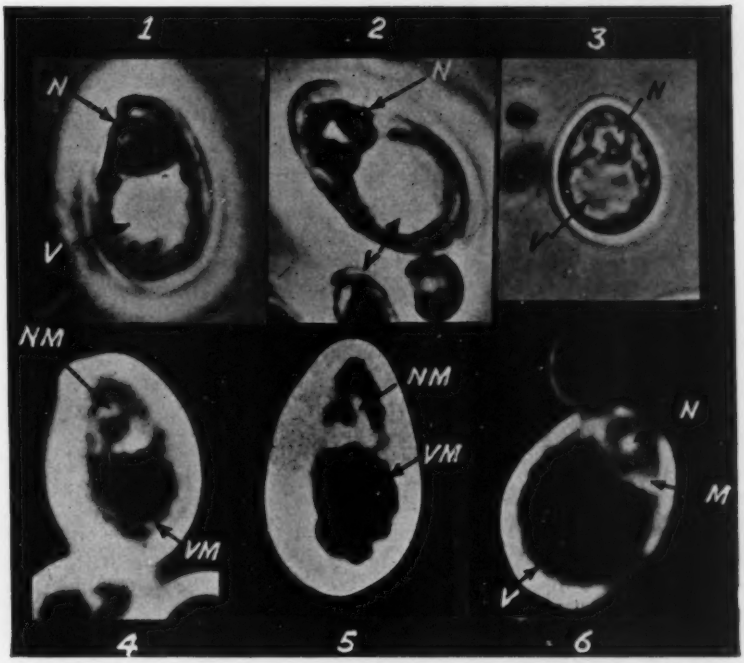
PHOTOS 1 and 2. Living cells under phase contrast.

PHOTO 3. Living cells under ordinary illumination.

PHOTOS 4, 5 and 6. Living cells under dark ground illumination.

N, Nucleus; *V*, Vacuole; *NM*, Nuclear Membrane; *VM*, Vacuolar Membrane.

Range in cell size 4–6 μ × 6–8 μ.


1
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CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGICAL ANATOMY OF SOME INDIAN HYDROPHYTES

III. The Stem of *Cleome chelidonii* Linn.

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(Communicated by Dr. T. S. Sabnis, F.A.SC.)

Introduction

Cleome chelidonii Linn., a member of the family Capparidaceæ, is an annual that usually grows in ponds and on the margins of lakes.

The stem of this amphibious species is tall and erect. It is succulent and green. The lower part of the stem is under water. The tall and erect form of the stem is very characteristic of the vegetation of the fresh-water swamps. It is a clear adaptation to the specialized conditions of the habitat, as a "tall slender shoot bends easily to breeze and elastically recovers" (Warming, 1925).

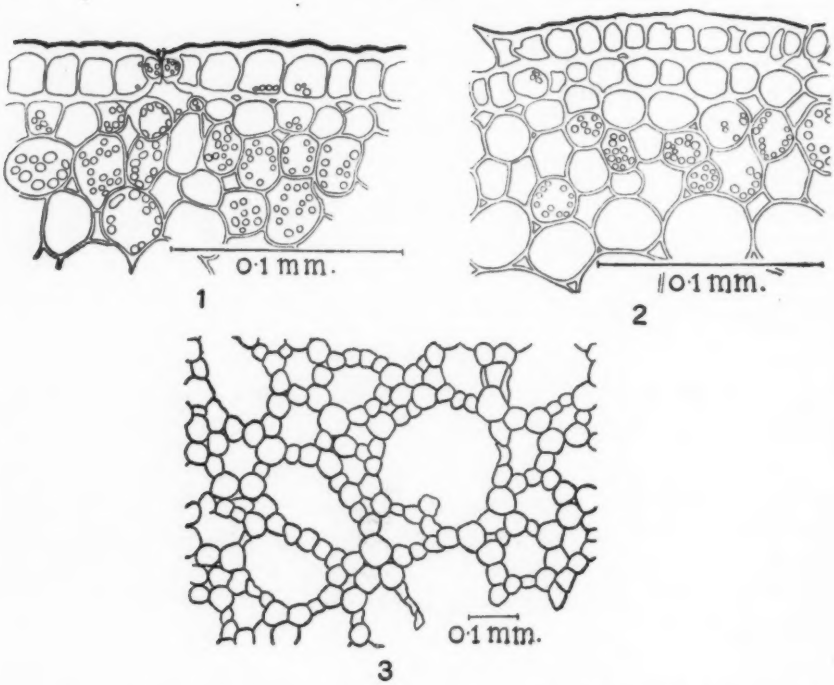
Description

1. *Epidermis.*—A single layer of thick-walled cells with thickening of cellulose which is more pronounced on the outer walls that are cuticularized (Fig. 1). The stomata lie almost in level with the general epidermal cells and are quite abundant.

2. *Cortex.*—The cortex forms a definite photosynthetic tissue of four to five layers of thin-walled parenchymatous cells containing chloroplasts (Fig. 1). There is a single row of seven to eight thick-walled hypodermal cells situated below the ridges in the slightly wavy outline of the stem (Fig. 2) there being no continuous zone of collenchyma below the epidermis. The endodermis is a distinct layer of large, barrel-shaped clear cells.

3. *Pith.*—This is the most characteristic region of the stem. It consists of an extensive parenchymatous tissue which is entirely and profusely lacunar, being traversed by a large number of schizogenous air-spaces and thereby presenting a net-like appearance (Fig. 3). The pith cells abound in starch grains.

4. *Vascular System.*—This is of the *Helianthus annuus* type. Secondary growth in thickness is normal.



TEXT-FIGS. 1–3. *Cleome chelidonii* Linn.—Fig. 1. Part of the T.S. of the stem showing the stoma, the thick-walled and cuticularized epidermis and the chlorenchymatous cortex. Note the absence of any well-developed collenchyma. Fig. 2. Part of the T.S. of the stem showing the hypodermal row of thick-walled cells. Fig. 3. Part of the T.S. of the stem showing the large schizogenous air-spaces in the lacunar pith.

DISCUSSION

1. *Lacunar Pith and its Physiological Significance.*—The presence of a well-developed lacunar tissue forming the pith is a special character of the stem of this hydrophyte. Arber (1920) points out that in water plants the cortex, which is generally prominent, shows the greatest development of air-spaces. Mullan (1933) concludes similarly that in the stems of plants of the salt swamps the inner cortex, as a rule, develops schizogenous lacunæ. The author (1954, 1955) also finds that in the two composites, *Cæsulia axillaris* Roxb. and *Eclipta alba* Hassk., it is the cortex of the stem that is lacunar. It is further interesting to observe that in another species of the same genus, *Cleome spinosa* Jacq., aerenchyma arises in the innermost layer of the primary cortex of the lower portion of the stem in specimens from swampy habitats (Metcalfe and Chalk, 1950).

The physiological significance of the extensive lacunar pith in the stem of *Cleome chelidonii* Linn. can hardly be overemphasized. It provides for efficient aeration without impairing the rigidity of the tall erect stem. Arber (1920) rightly observes that where the air-spaces are confined to the middle cortex, the outer cortex in such cases consists of elements that form a firmer shell of collenchyma. This need is eliminated in the stem of this species and the hypodermis is thickened only just below the ridges. There is, thus, an economy of material and the cortex is free to develop as an efficient photosynthetic tissue. The lacunar pith situated in the centre of an erect cylindrical organ like the stem does not reduce its mechanical rigidity which rests on the development of a compact peripheral cortical tissue that is further strengthened by a ring of stereid bundles in the pericycle.

The presence of abundant starch grains in the pith cells indicates that it functions also as a storage tissue.

2. The epidermal cells showing prominent thickening on the outer walls, the presence of a cuticle and the development of a definite photosynthetic tissue in the form of the chlorenchymatous cortex are anatomical characters of the stem in response to the aerial conditions to which it is exposed. The air is warm and usually there is a strong breeze. The leaflets of the compound leaf are comparatively small and their function of assimilation is shared by the green stem.

3. The mechanical system of the stem is well developed in the form of the thick-walled epidermis, local patches of thick-walled hypodermal cells, a compact peripheral cortex and a ring of stereid bundles. The vascular bundles arranged in the form of a ring and undergoing secondary growth form an efficient conducting system. As Weaver and Clements (1938) observe, these last two anatomical features of the stem of this amphibious plant enable it to grow erect.

5. *Phylogenetic Significance.*—The foregoing account brings out the hydrophytic nature of a species that belongs to the family Capparidaceæ, a comparatively primitive family of the dicotyledonous Angiosperms which are essentially a terrestrial race. This family shows a distribution in the warmer and drier parts of the world and includes some xerophytic members like *Capparis decidua* (Forsk.) Pax. (*C. aphylla* Roth.). It is significant, therefore, to find marsh plants, like the species described above, in this family which otherwise prefers warm and dry situations and further to discover the potentialities in its anatomical structure for a marked adaptation to a watery habitat. The development of a physiologically more advantageous tissue in the form of the lacunar pith in the stem of a comparatively primitive angio-

sperm in an attempt to adjust itself to a watery environment is a significant phenomenon in the ecological evolution of the Angiosperms.

ACKNOWLEDGEMENT

The author is deeply indebted to Dr. T. S. Sabnis, D.SC., F.A.SC., for kindly going through the manuscript of this paper and making valuable suggestions.

LITERATURE CITED

Arber, A. .. *Water Plants*, Cambridge, 1920.

Metcalfe, C. R. and Chalk, L. *Anatomy of the Dicotyledons*, Oxford, 1950.

Mirashi, M. V. .. "Contribution to Our Knowledge of the Physiological Anatomy of Some Indian Hydrophytes, I. The Stem of *Cæsulia axillaris* Roxb.," *Proc. Ind. Acad. Sci.*, 1954, **39** (3), 114–17.

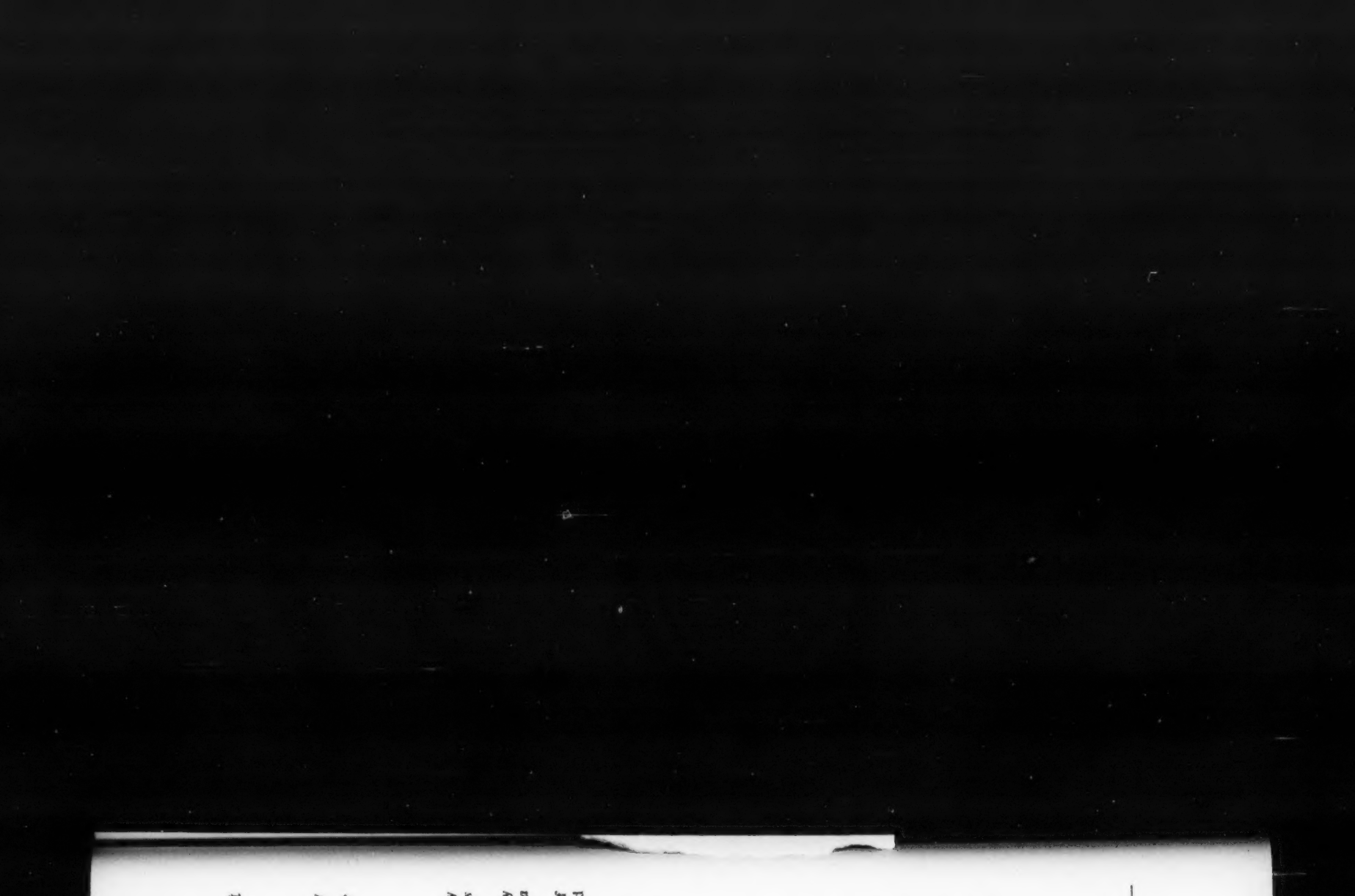
———— .. "Contribution to Our Knowledge of the Physiological Anatomy of Some Indian Hydrophytes, II. The Stem of *Eclipta alba* Hassk." *Ibid.*, 1955, **41** (4), 167–72.

Mullan, D. P. .. "Observations on the Biology and Physiological Anatomy of Some Indian Halophytes," *J. Indian Bot. Soc.*, 1932, **11**, and 1933, **12**.

Warming, E. .. *Oecology of Plants*, Engl. Trans., London, 1925.

Weaver, J. E. and Colments, F. E. *Plant Ecology*, New York and London, 1938.

426–56 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.



5

OBSERVATIONS ON THE FLORA OF YELLAPUR IN NORTH KANARA

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Received February 18, 1956

(Communicated by Dr. E. K. Janaki Ammal)

Introduction

According to Hooker (1855) the flora of India " is more varied than that of any country of equal area in the Eastern Hemisphere if not the whole Globe ". Whether India is richer in number of genera and species than any other area of equal dimensions is doubtful. India has less endemic genera and species than countries like China, Australia and S. Africa. A genus or even a species in India is often found distributed over a wide area and sometimes in widely separated areas. It is of the utmost importance for the understanding of the evolution of the flora of India to study the same species collected from different regions and different ecological habitats, to see if any genetic difference exists in geographical varieties of the same species. This can be done by making intensive studies of a particular genus from different parts of India or by analysis of samples of flora from different habitats in India. The present paper summarizes observations made in the Yellapur area during occasional visits spread over a period of two years when I served as Forest Botanist to the State of Bombay. The cytological data referring to the flora will be dealt with later.

The town of Yellapur is 1,200 ft. above sea-level. The heaviest rainfall occurs in the months of June, July and August with, perhaps, stray showers in October. Thus there is a prolonged dry season for 7 months when there is little or no rain. The total rainfall from June 1952 to May 1953 was 91 inches and from June 1953 to May 1954, 128 inches.* In spite of the prolonged dry season the existing semi-evergreen forests do not appear to have deteriorated except where trees have been deliberately felled or lopped for making room for cultivation or for any other purpose.

The present observations on the flora include the town proper and extend to the three neighbouring semi-evergreen forests, *viz.*, Angod " Kan ", 3 miles to the north-west, Telgeri " Kan ", 3 miles to the north-east, and Sahasrahalli " Kan ", practically adjoining the town south-eastwards. The

* Figures obtained from the Mamlatdar's Office at Yellapur.

observations also extend south-westwards along the Karwar road for a distance of 2 miles. The town of Naikainkeri, 1½ miles from Yellapur along the Karwar road is occasionally mentioned. The other three main roads leading away from Yellapur are the Sirsi and Mungod roads passing through Sahasrahalli Kan and the Hubli road passing through Telgeri Kan. During the first few months of 1953, the Silviculturist to Bombay State laid out a linear tree increment plot (L.T.I. Plot) in Telgeri Kan. Frequent references are made to the numbered trees in the plot.

Almost all the plant species listed below have been observed personally by me in the field and collected on the spot. It will be seen from the list that the trees, shrubs and woody climbers are better represented than the herbs since they are of greater interest from the forester's point of view.

Every attempt has been made to bring the nomenclature up-to-date and the Rev. Santapau's Flora[10] has been particularly useful in this connection. The descriptions of the listed species are usually to be found in Cooke[6] or Talbot[11] though the names used by these authors are here sometimes cited as synonyms. Where the description of any species is not to be found in either of the above works a cross-reference is made to the relevant publication where the description is to be found. Such cross-references are given in brackets after the name of the listed species.

Most of the species included in the list below are referred to a herbarium sheet number preceded by the collector's name. The exclamation mark after the herbarium sheet number indicates that the species has been satisfactorily compared with a specimen in the Dehra Dun Herbarium. The exclamation mark after a grass specimen number indicates that the specimen has been satisfactorily compared with material identified by Dr. N. L. Bor of Kew or Shri M. B. Raizada of Dehra Dun.

OBSERVATIONS

RANUNCULACEÆ

Naravelia zeylanica DC. Roadside, 1 mile from town along Mungod Road, in flower (15–11–1953).

Patil 2714 !

DILLENIACEÆ

Dillenia pentagyna Roxb. Angod Kan, leafless, but with flower buds (25–2–1955); near junction of Hubli and Haliyal Roads in Telgeri Kan, leafless, but with flowers (26–2–1955); Nos. 73, 77, etc., in the L.T.I. Plot at Telgeri.

Patil 2973!

ANNONACEÆ

Polyalthia fragrans Bedd. Telgeri Kan, in fruit (8–4–1955). Common. Nos. 11 and 75 in the L.T.I. Plot at Telgeri.

Patil 2990!

MENISPERMACEÆ

Cyclea sp. Telgeri Kan (9–10–1954).

Diploclisia glaucescens Diels, synonym, *Cocculus macrocarpus* Wight and Arn. Sahasrahalli Kan, in fruit (11–8–1953).

Patil 2571!

Stephania hernandifolia Walp. Sahasrahalli Kan, in flower (7–10–1954) and in fruit (8–10–1954). Occurs in the forest proper and in cleared space by the side of Mungod Road.

Patil 2914! 2929!

PAPAVERACEÆ

Argemone mexicana Linn. In town, in a ditch near the pond, with flowers and fruit (26–2–1955).

Patil 2979!

CAPPARIDACEÆ

Gynandropsis speciosa DC. (Bailey, *Standard Cyclopedia of Horticulture*, **2,** 1421). In open space near hut and rice field in Sahasrahalli Kan, with flowers and fruit (8–10–1954).

FLACOURTIACEÆ

Flacourtia montana Grah. Roadside, about 2 miles from town along Hubli Road, with young fruit (28–1–1954); No. 62 in L.T.I. Plot, Telgeri, with young fruit (29–1–1954); roadside in Angod Kan, near school, with ripe fruits (25–2–1955); hill behind Forest Bungalow No. 1, with ripe fruit (28–2–1955).

Patil 2724 ! 2736 ! 2964 !

Hydnocarpus laurifolia Sleumer, synonym, *H. wightiana* Blume. Sahasrahalli Kan, in fruit (5–5–1953).

GUTTIFERÆ

Garcinia indica Choiss. Sahasrahalli Kan, in fruit (6–5–1953); stray trees about 1 mile from town along Hubli and Mungod Roads; Angod Kan.

Patil 2534 !

Garcinia xanthochymus Hk. f. Sahasrahalli Kan, in fruit (6–5–1953, 2–2–1954). Somewhat gregarious where it occurs.

Patil 2752 !

Mesua ferrea Linn. In town, in flower (27–2–1955).

Patil 2982 !

DIPTEROCARPACEÆ

Vateria indica Linn. Roadside, near Yellapur bus stand, in fruit (5–5–1953) and flower (28–1–1954); grounds of Forest Bungalow No. 1; along Karwar Road, leading away from town.

MALVACEÆ

Sida acuta Burm. Near a hut by paddy fields in Sahasrahalli Kan, in flower (8–10–1954).

Sida rhombifolia Linn. var. *retusa* Masters. Side of Mungod Road, near town, in flower (8–10–1954). Common.

Urena sinuata Linn. Border of paddy field in Sahasrahalli Kan, in flower (7–10–1954).

Patil 2909!

BOMBACEÆ

Salmalia malabarica Schott and Endl., synonym, *Bombax malabaricum* DC. In town, side of Hubli Road, leafless, but in flower (28–1–1954, 25–2–1955).

STERCULIACEÆ

Helicteres isora Linn. Border of paddy field in Sahasrahalli Kan, in flower (7–10–1954); side of Haliyal Road in Telgeri Kan, in flower (9–10–1954); side of road to Angod Kan, in flower (12–10–1954).

Patil 2908!

Sterculia guttata Roxb. Near junction of Hubli and Haliyal Roads in Telgeri Kan, in flower, fruit and leaf (28–1–1954); No. 1 in L.T.I. Plot at Telgeri, in flower, fruit and leaf (29–1–1954).

Patil 2593! 2727!

TILIACEÆ

Grewia disperma Rottl., synonym, *G. lævigata* Talbot, **1**: 164. In an open site in Telgeri Kan, with flower and fruit (12–11–1953).

Patil 2708!

Grewia microcos Linn. Side of Hubli Road in Telgeri Kan, in flower and fruit (2–7–1953).

Patil 2556!

Grewia tilæfolia Vahl. Roadside in Angod Kan, near school (25–2–1955).

Grewia umbellifera Bedd. Sahasrahalli Kan, in fruit (10–8–1953); Angod Kan, in fruit (14–11–1953).

Patil 2568! 2711!

Triumfetta bartramia Linn., synonym, *T. rhomboidea* Jacq. Side of Mungod Road, near Forest Bungalow No. 1, in flower (6–10–1954).

Patil 2901!

MALPIGHIACEÆ

Aspidopterys cordata A. Juss. In a clearing by the side of Hubli Road in Telgeri Kan, in flower (9–10–1954).

Patil 2932!

RUTACEÆ

Glycosmis pentaphylla Corr. Sahasrahalli Kan, in flower and fruit (31–1–1954). Abundant.

Patil 2746!

Murraya koenigii Spreng. Telgeri Kan; Nos. 142 and 131 in L.T.I. Plot at Telgeri (29–1–1954).

Paramignya monophylla Wight. Sahasrahalli Kan, in fruit (10–8–1953, 2–2–1954); near the grounds of Forest Bungalow No. 1 on the south-east side, in fruit (2–2–1954).

Patil 2570! 2754!

MELIACEÆ

Dysoxylum malabaricum Bedd., synonym, *D. glandulosum* Talbot, **1**: 233. Sahasrahalli Kan, in fruit (6–5–1953).

Patil 2533!

Heynea trijuga Roxb. No. 90 in L.T.I. Plot at Telgeri, in fruit (30–6–1953); side of Sirsi Road in Sahasrahalli Kan, in flower (26–2–1955) and in fruit (12–10–1954).

Patil 2541! 2978!

Melia composita Willd., synonym, *M. dubia* Talbot, **1**: 226. Side of Hubli Road, about 1½ miles from town, in fruit and leaf (3–7–1953); in fruit, flower and new leaf (28–1–1954).

Patil 2560! 2723!

OPILIACEÆ

Cansjera rheedii Gmel. Roadside along the road to Angod Kan, in flower (12–10–1954); open space in Telgeri Kan, in fruit (1–2–1954).

Patil 2750! 2945!

CELASTRACEÆ

Celastrus paniculata Willd. Sahasrahalli Kan, in flower (10–8–1953) and in fruit (7–10–1954).

Patil 2569!

Lophopetalum wightianum Arn. Sahasrahalli Kan, in fruit (6–5–1953); about 2 miles from town along Hubli Road, in flower (28–1–1954).

Patil 2532!

RHAMNACEÆ

Zizyphus caracutta Roxb., synonym, *Z. xylopyra* var. *microcarpa* Talbot **1**: 298. Open space in Angod Kan, in fruit (13–8–1953).

Patil 2579!

Zizyphus œnoplia Mill. Near border of paddy field in Sahasrahalli Kan in flower (7–10–1954).

Patil 2912!

Zizyphus rugosa Lamk. Side of Karwar Road beyond the junction with the road to Magod, with a few leaves, flowers and young fruit (26–2–1955); border of paddy field in Sahasrahalli Kan (27–2–1955).

Patil 2980!

AMPELIDACEÆ

Cissus discolor Blume, synonym, *Vitis discolor* Dalz. Side of Mungod Road in Sahasrahalli Kan, in flower and fruit (6–10–1954); side of Hubli Road in Telgeri Kan, in flower and fruit (9–10–1954).

Patil 2903!

Leea indica Merrill, synonym, *L. sambucina* Willd. Roadside in Telgeri Kan, in flower and fruit (12–11–1953); roadside in Sahasrahalli Kan, in flower and fruit (15–11–1953); near school in Angod Kan, in flower (25–2–1955). Common.

SAPINDACEÆ

Sapindus trifoliatus Linn., synonym, *S. laurifolius* Vahl. Roadside in town, in flower (17–11–1953).

Patil 2718!

ANACARDIACEÆ

Anacardium occidentale Linn. In the grounds of Forest Bungalow No. 1, in flower (26–2–1955).

Buchanania lanzan Spreng., synonym, *B. latifolia* Roxb. Near town, on way to Telgeri, in flower (28–1–1954).

Patil 2722!

Holigarna arnottiana Hk. f. Near town, on way to Telgeri, in flower (28–1–1954). Sahasrahalli Kan, in fruit (5–5–1953).

Patil 2721!

Lannea grandis Engler, synonym, *Odina wodier* Roxb. Side of Mungod Road, not far from Forest Bungalow No. 1, leafless, but in flower (24–2–1955); side of pond at Naikainkeri, leafless, but in flower (26–2–1955); near paddy field in Sahasrahalli Kan, with a few old leaves, flowers and fruit (27–2–1955).

Patil 2959! 2985!

Mangifera indica Linn. Roadside in town, in fruit (6–5–1953) and flower (28–1–1954).

Nothopegia colebrookiana Bl. Telgeri Kan, in flower (29–1–1954); Nos. 27 and 32 in L.T.I. Plot at Telgeri.

Patil 2732!

Spondias mangifera Willd. Telgeri Kan, in leaf and fruit (2–7–1953); in town, leafless, but bearing flowers (27–2–1955).

Patil 2557! 2986!

PAPILIONACEÆ

Desmodium triquetrum DC. Near border of paddy field in Sahasrahalli Kan, in flower (7–10–1954).

Patil 2917!

Erythrina variegata Linn. var. *orientalis* Merrill, synonym, *E. indica* Lamk. Roadside in town, leafless, but in flower (28–1–1954, 25–2–1955).

Patil 2967!

CÆSALPINACEÆ

Bauhinia foveolata Dalz. Roadside near Forest Naka at Telgeri, in flower and leaf (11–11–1953) and in fruit and leaf (28–1–1954).

Cæsalpinia coriaria Willd. (Benthal, *Trees of Calcutta*, p. 165). Grounds of Forest Bungalow No. 1, in fruit (5–5–1953, 26–2–1955).
Patil 2976!

Cæsalpinia mimosoides Lam. Roadside, 1 mile from town along Mungod Road (31–1–1954).
Patil 2747!

Cassia fistula Linn. Grounds of Forest Bungalow No. 1, in leaf and fruit (10–11–1953), leafless, but in flower (26–2–1955).
Patil 2970!

Cassia leschenaultiana DC. (Gamble, *Flora of Madras Presidency*, **3**: 403). This plant is often regarded as a variety of *Cassia mimosoides*. The Yellapur plant matches with those specimens of the latter species at Dehra Dun that have minute pinnæ.
Patil 2920!

Cassia sophera Linn. Waste ground near P.W.D. Bungalow, in flower and fruit (14–10–1954).
Patil 2951!

Cassia tora Linn. Near Forest Bungalow No. 1, in flower and fruit (6–10–1954).
Patil 2907!

MIMOSACEÆ

Entada phaseoloides Merrill, synonym, *E. scandens* Benth. Roadside, near junction of Hubli and Haliyal Roads in Telgeri Kan, in leaf and fruit (11–11–1953), with flowers and new leaf (26–2–1955).
Patil 2975!

Enterolobium saman Prain (Benthall, *Trees of Calcutta*, p. 224). Side of Hubli Road in town, with flowers (25–2–1955).
Patil 2966!

Mimosa pudica Linn. Roadside in Telgeri Kan, in flower and fruit (8–8–1953).
Patil 2561!

Xylia xylocarpa Taub., synonym, *X. dolabriformis* Benth. Angod Kan, in fruit (13–8–1953); Sahasrahalli Kan, in fruit (12–10–1954).

Patil 2580!

RHIZOPHORACEÆ

Carallia brachiata Merrill, synonym, *C. lucida* Roxb. Roadside, about 1 mile from town along Mungod Road, in flower (11–8–1953); Angod Kan, near school, in flower (25–2–1955).

Patil 2575!

COMBRETACEÆ

Calycopteris floribunda Lamk. Roadside near Telgeri Forest Naka, in flower (30–1–1954); Angod Kan, at the edge of the clearing near school, in flower (25–2–1955).

Patil 2743! 2961!

Terminalia belerica Roxb. Side of Haliyal Road in Telgeri Kan, in fruit (9–10–1954).

Terminalia chebula Retz. Side of Sirsi Road, near town, in fruit (7–8–1953).

Terminalia paniculata Roth. In Telgeri Kan, with young fruit (9–11–1953) and flowers (9–10–1954); roadside in Sahasrahalli Kan, in fruit (28–1–1954).

Patil 2594!

Terminalia crenulata Roth, synonym, *T. tomentosa* Talbot, **2**: 18. Roadside in Sahasrahalli Kan, with fruit, flower and new leaf (5–5–1953); side of Hubli Road, near town, in fruit (28–1–1954).

MYRTACEÆ

Syzygium caryophyllatum Alston, synonym, *Eugenia corymbosa* Lam. Open space off Mungod Road, about 1 mile from town, in fruit (11–8–1953).

Patil 2576!

Syzygium cumini Skeels, synonym, *Eugenia jambolana* Lamk. Roadside in Angod Kan, near school, in flower (25–2–1955).

Patil 2965!

LECYTHIDACEÆ

Careya arborea Roxb. Near Forest Bungalow No. 1, in leaf and fruit (29–6–1953); side of Hubli Road, in town, in flower, but leafless (25–2–1955).

Patil 2962!

MELASTOMACEÆ

Melastoma malabathricum Linn. Side of Karwar Road, near junction with the road to Magod (14–10–1954); border of paddy field in Sahasrahalli Kan, in flower (24–2–1955).

Patil 2948! 2957!

Memecylon talbotianum Brandis. Telgeri Kan, in fruit (2–7–1953); Angod Kan, in fruit (14–11–1953).

SAMYDACEÆ

Casearia rubescens Dalz. Sahasrahalli Kan, in fruit and flower (6–5–1953). The Yellapur plant is a tree, *vide* Talbot's description and Bor's South Indian specimens in Dehra Dun Herbarium.

Patil 2535!

ARALIACEÆ

Schefflera venulosa Harms, synonym, *Heptapleurum venulosum* Seem. Sahasrahalli Kan (8–10–1954); Angod Kan (12–10–1954).

Patil 2922!

RUBIACEÆ

Adina cordifolia Hook. f. Sahasrahalli Kan, in flower (16–8–1953).

Patil 2584!

Canthium dicoccum Merrill, synonym, *C. didymum* Talbot, **2:** 107. No. 93 in L.T.I. Plot at Telgeri, in flower (30–1–1954).

Patil 2741!

Chasalia curviflora Thw. Sahasrahalli Kan, in flower (29–6–1953); side of lane in Telgeri Kan, in fruit (9–10–1954).

Patil 2931!

Ixora brachiata Roxb. Angod Kan, in flower (13–8–1953, 25–2–1955); Sahasrahalli Kan, in flower (24–2–1955); Nos. 4, 9, 78, 80, 81, 84 and 100 in L.T.I. Plot at Telgeri, in flower (28–2–1954).

Patil 2578! 2761! 2762!

Ixora nigricans R. Br. Sahasrahalli Kan, in flower (24–2–1955); Angod Kan, near school, in flower (25–2–1955).

Patil 2956! 2968!

Meynia laxiflora Robyns, synonym, *Vangueria spinosa* Hk. f. *pro parte*. For description of this new species *vide* Santapau's *Flora of Khandala*,

p. 134. Side of Hubli Road in Telgeri Kan, in fruit (1–7–1953) and in flower (26–2–1955); No. 158, etc., in L.T.I. Plot, Telgeri.

Patil 2552! 2971!

Mitragyna parvifolia Korth., synonym, *Stephegyne parvifolia* Korth. Open space in Sahasrahalli Kan, in flower (6–5–1953).

Mussænda frondosa Linn. Roadside in Sahasrahalli Kan, in flower and fruit (11–8–1953); side of lane in Telgeri Kan, in fruit (9–10–1954).

Oldenlandia nitida Gamble, synonym, *Hedyotis nitida* W. & A. Roadside on way to Angod Kan, with flowers and young fruit (13–10–1954).

Patil 2949!

Pavetta indica Linn. Sahasrahalli Kan, in flower (6–5–1953).

Patil 2535 A!

Psychotria dalzellii Hk. f. Sahasrahalli Kan, in flower and fruit (11–8–1953); Telgeri Kan, in fruit (9–10–1954). Common.

Patil 2935!

Psychotria flavida Talbot. In a semi-evergreen patch 2 miles from town along the Karwar Road, in fruit (10–10–1954).

Randia brandisii Gamble (in *Flora of Madras*, p. 616, 1921), synonym, *R. dumetorum* Lamk. *pro parte*. Roadside in Sahasrahalli Kan, in flower and fruit (6–5–1953); roadside in Telgeri Kan, in fruit (1–7–1953). Common.

Patil 2544!

Rubia cordifolia Linn. Side of Haliyal Road in Telgeri Kan, in flower (9–10–1954); Sahasrahalli Kan (8–10–1954).

Patil 2936! 2923!

Wendlandia notoniana Wall. Border of paddy field in Sahasrahalli Kan, in flower (24–2–1955).

Patil 2960!

COMPOSITÆ

Ageratum conyzoides Linn. Border of paddy field in Sahasrahalli Kan, in flower (7–10–1954).

Patil 2910!

Elephantopus scaber Linn. Under trees near Forest Bungalow No. 1, in flower (6–10–1954); under trees by the side of the road to Angod Kan, in flower (12–10–1954).

Patil 2906!

Gynura angulosa DC. Near border of paddy field in Sahasrahalli Kan, in flower and fruit (7–10–1954).

Patil 2911!

Spilanthes acmella Murr. Side of Mungod Road, near Forest Bungalow No. 1, in flower (6–10–1954).

Patil 2905!

MYRSINACEÆ

Ardisia solanacea Roxb., synonym, *A. humilis* Talbot, **2:** 149. Sahasrahalli Kan, in flower (6–5–1953) and in fruit (14–10–1954).

Patil 2952!

Embelia tsjeriam-cottam DC., synonym, *E. robusta* Talbot, **2:** 147. Open spaces in Sahasrahalli Kan, in flower and fruit (16–8–1953). Common.

Patil 2585!

SAPOTACEÆ

Mimusops elengi Linn. Sahasrahalli Kan, in flower (5–5–1953); side of Mungod Road about 1 mile from town, in fruit (11–8–1953).

Patil 2532! 2574!

Pouteria tomentosa Bæhni, synonym, *Sideroxylon tomentosum* Roxb. Side of Mungod Road, about 1 mile from town, in fruit (15–11–1953), in fruit and flower (2–2–1954); hillock overlooking the pond at Naikainkeri, in fruit (10–10–1954).

Patil 2573! 2716!

EBENACEÆ

Diospyros microphylla Bedd. Sahasrahalli Kan, with female flowers and fruit (6–5–1953). Telgeri Kan.

Patil 2531!

Diospyros montana Roxb. Side of Hubli Road in Telgeri Kan, in fruit (1–7–1953); more than a mile from town along Hubli Road, in fruit (17–11–1953).

Patil 2551!

SYMPLOCACEÆ

Symplocos spp. Nos. 6 and 23 in the L.T.I. Plot, Telgeri, belong to this genus. They are two distinct species. No. 23 appears to be *Symplocos beddomei* C. B. Clarke.

OLEACEÆ

Jasminum malabaricum Wight. Hill behind Forest Bungalow No. 1, with flowers (28–2–1955).

Patil 2987!

Olea dioica Roxb. Side of Mungod Road about 1 mile from town, with male flowers (11–8–1953); Nos. 38 and 60 in the L.T.I. Plot at Telgeri. Well represented in Sahasrahalli, Telgeri and Angod Kans. Patil 2572! 2597! 2600!

APOCYNACEÆ

Alstonia scholaris R. Br. Side of Hubli Road, near town (8–8–1953); side of Mungod Road, in Sahasrahalli Kan (7–10–1954).

Carissa congesta Wight, synonym, *C. carandas* Cooke, **2**: 124. Open space in Telgeri Kan, off Hubli Road, in flower (1–2–1954).

Ervatamia heyneana Cooke, synonym, *Tabernæmontana heyneana* Wall. Roadside in Sahasrahalli Kan, with fruit (1–7–1953); hill behind Forest Bungalow No. 1, in flower (28–2–1955).

Patil 2543! 2988!

Rauwolfia densiflora Benth. Sahasrahalli Kan, in fruit (10–8–1953). On the date cited it was gregarious in the Kan in a spot between Mungod Road and paddy fields.

Patil 2567!

Rauwolfia serpentina Benth. Side of lane in Telgeri Kan, in flower (9–10–1954).

Thevetia peruviana Merr. (Benthall, *Trees of Calcutta*, p. 302). In the clearing behind the school in Angod Kan, with flowers (25–2–1955).

ASCLEPIADACEÆ

Asclepias curassavica Linn. (Hooker, *Flora of Br. India*, **4**: 18). Near Forest Bungalow No. 1, in flower (14–10–1954).

Patil 2950!

Hemidesmus indicus Br. Open space near paddy fields in Sahasrahalli Kan, in flower (7–10–1954).

Patil 2915!

LOGANIACEÆ

Strychnos nux-vomica Linn. Telgeri Kan, in leaf and fruit (8–8–1953); behind Forest Bungalow No. 1, with new leaf and fruit (25–2–1955).

GENTIANACEÆ

Canscora pauciflora Dalz. Roadside along Mungod Road, near Forest Bungalow No. 1, in flower (7–10–1954).

Patil 2916!

Exacum petiolare Griseb. (Gamble, *Flora of Madras*, p. 874, 1923). Roadside along Mungod Road, about one mile from town, in flower (8–10–1954).

Patil 2925!

BORAGINACEÆ

Ehretia canarensis Miq., synonym, *E. lævis* Roxb. var. *canarensis* C. B. Clarke. Near school in Angod Kan, with flowers (14–11–1953); side of Mungod Road in Sahasrahalli Kan, with flowers and fruit (15–11–1953); near a hut adjoining paddy fields in Sahasrahalli Kan, in flower (8–10–1954).

Patil 2715! 2709!

CONVOLVULACEÆ

Argyreia pilosa Wight & Arn. In shady places near Forest Bungalow No. 1, in flower (10–10–1954).

Patil 2953!

SOLANACEÆ

Solanum denticulatum Blume. Side of Hubli Road in Telgeri Kan, in flower (8–8–1953).

Solanum ferox Linn. In the clearing near the school in Angod Kan, in flower and fruit (12–10–1954).

Patil 2944!

Solanum giganteum Jacq. Sahasrahalli Kan, in flower and fruit (31–1–1954); near school in Angod Kan, in fruit (25–2–1955).

Patil 2745!

Solanum torvum Swartz (Gamble, *Flora of Madras*, p. 937, 1923). Near paddy field in Sahasrahalli Kan, in flower and fruit (13–10–1954).

Patil 2947!

SCROPHULARIACEÆ

Scoparia dulcis Linn. (Hooker, *Flora of Br. India*, **4**: 289). Roadside near Forest Bungalow No. 1, in flower and fruit (6–10–1954).

Patil 2904!

ACANTHACEÆ

Adhatoda vasica Nees. Hedges in town, in flower (14–10–1954, 28–2–1955).
Patil 2989!

Asteracantha longifolia Nees. Near paddy field in Sahasrahalli Kan, in flower (8–10–1954).

VERBENACEÆ

Callicarpa tomentosa Murray, synonym, *C. lanata* Linn. Roadside in Sahasrahalli Kan, in flower and fruit (3–2–1954); border of paddy field in Sahasrahalli Kan, in flower (24–2–1955). Common.
Patil 2958!

Clerodendron serratum Moon: Talbot, **2**: 359. Roadside in Telgeri Kan, in flower (8–8–1953).
Patil 2562!

Clerodendron viscosum Vent., synonym, *C. infortunatum* Talbot **2**: 358. Telgeri Kan and Sahasrahalli Kan, in flower (8–8–1953) and in fruit (8–10–1954). Gregarious.
Patil 2566!

Gmelina arborea Roxb.: Talbot, **2**: 348. By the side of the Gaodevi temple in town, with flowers and young fruit (27–2–1955).
Patil 2981!

Lantana camara Linn. var. *aculeata* Moldenke, synonym, *L. aculeata* Linn. Hill behind Forest Bungalow No. 1, in flower throughout the year. Common.

Premna coriacea Clarke. Climbing on *Lannea grandis* Engler between the pond and the road at Naikainkeri, in leaf (10–10–1954) and with new leaf and flower buds (26–2–1955).
Patil 2937!

Vitex altissima Linn. f. Telgeri Kan, in flower (8–4–1955); Nos. 183, 163, etc., in the L.T.I. Plot at Telgeri; Sahasrahalli Kan.
Patil 2994!

Vitex negundo Linn. Hedges in town, in flower and fruit (27–2–1955).
Patil 2984!

LABIATÆ

Leucas biflora R. Br. Open space in Sahasrahalli Kan, near paddy field, in flower (7–10–1954).

Patil 1913!

Leucas ciliata Benth. Side of Mungod Road, about 2 furlongs from Forest Bungalow No. 1, in flower (6–10–1954).

Patil 2902!

POLYGONACEÆ

Polygonum chinense Linn. var. *ovalifolium* Meissn. Border of paddy field in Sahasrahalli Kan, in flower (7–10–1954).

Patil 2919!

PIPERACEÆ

Piper nigrum Linn. Sahasrahalli Kan, in fruit (8–10–1954).

Patil 2921!

MYRISTICACEÆ

Myristica malabarica Lamk. No. 17 in the L.T.I. Plot at Telgeri, in fruit (3–2–1954); male tree in Telgeri Kan, in flower (3–2–1954); Nos. 8 and 28 in the L.T.I. Plot.

Patil 2536! 2756!

Knema attenuata Warb., synonym, *Myristica attenuata* Wall. Nos. 21, 69 and 96 in L.T.I. Plot at Telgeri, in fruit (29–6–1953); male tree in Sahasrahalli Kan, in flower (2–2–1954); No. 88 in the L.T.I. Plot.

Patil 2537!

LAURACEÆ

Actinodaphne hookeri Meissn. Telgeri Kan, in flower (9–11–1953); roadside on way to Angod Kan, in flower (12–10–1954). The Yellapur plant resembles *A. angustifolia* Nees from Burma in the Dehra Dun Herbarium with regard to length and shape of leaf, the main veins and the degree of pubescence but differs from the latter in that the minute venation is more distinct.

Patil 2586 ! 2587 ! 2946 !

Cinnamomum zeylanicum Blume. Telgeri Kan, in flower (28–1–1954).

Patil 2725 ! 2744 !

Cryptocarya wightiana Thw. Sahasrahalli Kan, with flowers and young fruit (5–5–1953, 6–5–1953).

Patil 2528! 2529!

Litsea chinensis Lamk., synonym, *L. sebifera* Pers. Side of Hubli Road, over a mile from town, in flower (3–7–1953) and in fruit (15–8–1953).

Patil 2559! 2583!

Litsea coriacea Hk. f. (Gamble, *Flora of Madras*, p. 1236, 1925). Telgeri Kan, with flowers (9–11–1953) and young fruits (29–1–1954); Nos. 33, 35, 43, 52, 66, 71 and 178 in the L.T.I. Plot, Telgeri.

Patil 2590! 2591! 2592! 2595! 2701! 2734!

Machilus macrantha Nees. Side of Haliyal Road in Telgeri Kan, in flower (11–12–1954).

Giriyappanavar 2954!

ELÆAGNACEÆ

Elæagnus latifolia Linn. Near school in Angod Kan, with flowers (14–11–1953); hill behind Forest Bungalow No. 1, in flower and fruit (4–2–1954).

Patil 2710! 2755!

LORANTHACEÆ

Helicanthes elastica Dans., synonym, *Loranthus elasticus* Desr. Roadside in town, on mango tree, with flowers and fruit (11–10–1954).

Patil 2942!

Viscum angulatum Heyne. On roadside tree, 2 miles from town along Karwar Road, in fruit (10–10–1954).

Patil 2938!

EUPHORBIACEÆ

Aporosa lindleyana Baill. Side of Mungod Road, one mile from town, with female flowers (27–1–1954); No. 2 in the L.T.I. Plot at Telgeri, with female flowers (29–1–1954); Telgeri Kan, in fruit (8–4–1955).

Patil 2720! 2730! 2993!

Bridelia scandens Willd., synonym, *B. stipularis* Talbot, **2**: 437. In a clearing off Mungod Road, over a mile from town, in flower (15–11–1953).

Patil 2717!

Cleidion spiciflorum Merrill, synonym, *C. javanicum* Bl. Telgeri Kan, in fruit (8–4–1955).

Patil 2992!

Emblica officinalis Gaertn., synonym, *Phyllanthus emblica* Linn. Side of Mungod Road, about one mile from town, with young fruit (11–8–1953).

Patil 2577!

Glochidion johnstonei Hk. f. Side of Hubli Road in Telgeri Kan, with male and female flowers and fruit (26–2–1955).

Macaranga peltata M. Arg., synonym, *M. tomentosa* Wight. Off Hubli Road in Telgeri Kan, with flower buds (28–1–1954).

Mallotus albus M. Arg. Side of Haliyal Road in Telgeri Kan, in flower (9–11–1953); in a clearing in Telgeri Kan, with fruit (12–11–1953); near Forest Bungalow No. 1 in flower (9–11–1953); border of paddy field in Sahasrahalli Kan, in flower (8–10–1954). Not common.

Patil 2588! 2707!

Mallotus philippinensis M. Arg. Off Hubli Road, near town, in flower (14–11–1953); side of lane in Telgeri Kan, with fruit (1–2–1954); Angod Kan (14–11–1953).

Patil 2712! 2749!

Mallotus repandus M. Arg. Side of Haliyal Road in Telgeri Kan (9–10–1954); in Sahasrahalli Kan (10–10–1954); roadside on way to Angod Kan (12–10–1954).

Patil 2933!

Melanthesa turbinata Wight, synonym, *Breynia patens* Rolfe. Telgeri Kan, in flower and fruit (2–7–1953).

Patil 2558!

ULMACEÆ

Celtis cinnamomea Lindl. Side of Hubli Road in Telgeri Kan, near junction with Haliyal Road, in fruit (26–2–1955).

Patil 2977!

Trema orientalis Blume. Roadside in Telgeri Kan, in flower (9–11–1953).

Patil 2589!

MORACEÆ

Antiaris toxicaria Leschen. Telgeri Kan: between Telgeri depot and L.T.I. Plot, 1½ furlongs from depot and 100 yards from the road,

with profuse male receptacles (11–10–1954); No. 36 in the L.T.I. Plot, Telgeri.

Patil 2940! 2943!

Artocarpus hirsuta Lamk. Sahasrahalli Kan, with ripe fruit (5–5–1953); Telgeri Kan, with ripe fruit (8–4–1955).

Patil 2995!

Artocarpus integra Merrill, synonym, *A. integrifolia* Linn. f. Side of Hubli Road, in town, with male inflorescence (28–1–1954).

Patil 2728!

Artocarpus lakoocha Roxb. Sahasrahalli Kan, with ripe fruit (5–5–1953); side of Haliyal Road in Telgeri Kan, with female inflorescence (29–1–1954).

Patil 2527! 2738!

Ficus asperrima Roxb. Roadside in Telgeri Kan, with receptacles (2–7–1953, 28–1–1954).

Patil 2555! 2726!

Ficus bengalensis Linn. In town, side of Hubli Road.

Ficus glomerata Roxb. In town, off the road leading to Siddiq Shahwali's tomb, with ripe receptacles (27–2–1955).

Patil 2983!

Ficus mysorensis Heyne. Side of Hubli Road, in town, with receptacles (28–1–1954).

Patil 2729!

Ficus nervosa Roth. No. 42 in L.T.I. Plot at Telgeri, with receptacles (29–6–1953).

Patil 2538!

Ficus religiosa Linn. Near petrol pump and other places in town.

Ficus tsjakela Burm. Telgeri Kan, with receptacles (8–8–1953, 17–11–1953); side of Sirsi Road in Sahasrahalli Kan, with receptacles (26–2–1955).

Patil 2564! 2719! 2974!

CASUARINACEÆ

Casuarina equisetifolia Linn.: Talbot, **2**: 536. Grounds of Forest Bungalow No. 1, in flower and fruit (26–2–1955).

Patil 2972!

GNETACEÆ

Gnetum ula Brongn., synonym, *G. scandens* Talbot, **2**: 543. Hill behind Forest Bungalow No. 1, in fruit (14–8–1953); edge of the evergreens overlooking paddy fields in Sahasrahalli Kan, with flowering spike (2–2–1954).

Patil 2582! 2753!

HYPOXYDACEÆ

Curculigo orchioides Gaertn. In Sahasrahalli Kan, near Forest Bungalow No. 1, in flower (8–10–1954).

Patil 2924!

DIOSCOREACEÆ

Dioscorea hispida Dennst., synonym, *D. dæmona* Roxb. Side of Hubli Road, about one mile from town, in flower (9–8–1953).

Patil 2567 A!

LILIACEÆ

Chlorophytum orchidastrum Lindl. In L.T.I. Plot at Telgeri, in flower and fruit (9–10–1954).

Patil 2934!

SMILACEÆ

Smilax zeylanica Linn., synonym, *S. macrophylla* Roxb. Roadside clearing in Angod Kan, with flowers and fruit (13–8–1953).

Patil 2581!

PALMÆ

Areca catechu Linn. In a plantation in Sahasrahalli Kan, along with bananas (24–2–1955).

Cocos nucifera Linn. In town (26–2–1955).

Caryota urens Linn. Telgeri Kan, in flower (1–7–1953) and in fruit (11–11–1953); near school in Angod Kan (25–2–1955).

ARACEÆ

Arisæma tortuosum Schott. Telgeri Kan, in flower (9–8–1953).

Patil 2567 B!

Pothos scandens Linn. Sahasrahalli Kan, in flower (8–10–1954).

Patil 2927!

GRAMINEÆ

Arundinella lawii Hk. f. Border of paddy field in Sahasrahalli Kan in flower (7–10–1954).

Hugar 3110!

Echinochloa colona Link., synonym, *Panicum colonum* Linn. Border of paddy field in Sahasrahalli Kan, in flower (7–10–1954).

Hugar 3102!

Eragrostis unioloides Nees, synonym, *E. amabilis* Wight & Arn. Roadside in Sahasrahalli Kan, in flower (7–10–1954).

Hugar 3111!

Heteropogon contortus P. Beauv., synonym, *Andropogon contortus* Linn. Near paddy field in Sahasrahalli Kan, in flower (10–10–1954).

Hugar 3121!

Paspalidium flavidum A. Camus, synonym, *Panicum flavidum* Retz. In open space in Sahasrahalli Kan, in flower (7–10–1954).

Hugar 3106!

Themeda triandra Forsk., synonym, *T. imberbis* T. Cooke. Border of paddy field in Telgeri Kan, in flower (9–10–1954).

Hugar 3101!

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REFERENCES

1. Bailey, L. H. .. *The Standard Cyclopedia of Horticulture*, New York, 1933.
2. Beddome, R. H. .. *The Flora Sylvatica for Southern India*, Madras, 1869–74.
3. Benthall, A. P. .. *The Trees of Calcutta and its Neighbourhood*, Calcutta, 1946.
4. Blatter, E. and McCann, C. *The Bombay Grasses*, Delhi, 1935.
5. Bor, N. L. .. *Manual of Indian Forest Botany*, Bombay, 1953.
6. Cooke, T. .. *The Flora of the Presidency of Bombay*, London, 1901–08.
7. Gamble, J. S. .. *Flora of the Presidency of Madras*, London, 1915–36.
8. Hooker, J. D. .. *The Flora of British India*, London, 1872–97.
9. Parkinson, C. E. .. "Indian Terminalias of the section Pentaptera," *Indian Forest Records (New Series), Botany*, 1936, **1** (1).
10. Santapau, H. .. "The Flora of Khandala on the Western Ghats of India," *Records of the Botanical Survey of India*, 1953, **16** (1).
11. Talbot, W. A. .. *Forest Flora of the Bombay Presidency and Sind*, Poona, 2 vols., 1909, 1911.

SUNSCORCH IN ARECA

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The areca palm (*Areca catechu*) is cultivated in about 3,00,000 acres in India chiefly in the States of Madras, Mysore, Travancore-Cochin, Bombay, West Bengal and Assam. The bulk of the area is however in S. India. The palm is grown at different altitudes ranging from sea-level to about 2,500 feet. The cultivation methods vary in different tracts. In parts of S. Kanara and in Coimbatore Districts and in certain parts of Mysore State pure areca gardens are generally located in sheltered valleys where facilities for irrigation exist and where the palms are irrigated during the hot summer months. In some parts of Malabar District and in the State of Travancore-Cochin, areca is planted mixed with coconut, rubber, pepper, etc. Sometimes the palm is grown indiscriminately in exposed situations on the slopes of hills without provision for shade and without facilities for irrigation during the dry months.

The growth of the palm and the yield of nuts vary considerably in proportion to the attention bestowed and the maintenance of adequate soil moisture during the dry months. The best returns are obtained from well manured and irrigated gardens, while those growing on hill slopes where the soil is parched up for over four months of the year and where torrential rains wash down the soil during the monsoons, are usually poor in growth with thin stem and yellowish foliage producing small bunches and giving poor yields.

This palm is subject to several diseases, the chief among which are the fruit rot and bud rot (*Phytophthora palmivora*), wilt (*Ganoderma lucidum*) and 'band' (a physiologic disorder). In recent years another type of damage resulting in the formation of splits, cracks or depressions in the stem or cavities inside the stem, is reported to be becoming conspicuous. Such palms are easily broken during the gales associated with the monsoons. This kind of damage has been noticed by most of the growers from days of old and many of them had realised that it might be due to sunscorch. But some growers in S. Kanara were under the presumption that this type of damage was the result of the continuous use of Bordeaux mixture for the control of fruit rot. However this presumption could not be maintained as the same type of damage occurs in localities where this fungicide had never been used, as in Mettupalayam or Wynaad. The location of the affected portion always

between the western and southern sides of the stem proves that this assumption is not correct. Only palms which are exposed to the sun exhibit the damage.

Venkatarayan (1931) has reported the frequent occurrence of such damage in the Malnad areas of Mysore. Sundararaman and his co-workers (1928) have described a stem-bleeding disease of areca caused by *Ceratostomella paradoxa* in Malabar and Coimbatore. Later observations have shown that for all practical purposes this disease is noticed only on palms previously affected by sunscorch.

The damage caused by sunscorch is common in many gardens in Madras and Travancore-Cochin States. Both young and old palms are affected. Those growing in situations exposed to the sun during the afternoon are the ones involved. The damage is all on one and the same side, the south-western side of the stem. The drying and destruction of the tissues in the outer portions of the stem are noticeable for varying heights. In some, cavities are developed at the base and these extend upwards to a height of three or more feet from the ground. In others such cavities are noticed a few feet below the crown and continue to very near the base. In still others these occur in the middle portion. The extent of the damage appears to be due to the length of exposure of the tender portions to the sun and the ravages of secondary organisms. In young palms the exposed surface turns yellow and dries up. Fissures are formed in these portions and sooner or later cavities appear extending from the base upwards. These cavities are usually broader below and tapering above. The tissues are dark and the vascular bundles stick out as dark fibres. A dark fluid may sometimes be seen flowing out of these depressions. Usually this becomes evident when secondary infection by *C. paradoxa* has occurred. In the older palms also such depressions may develop. These may be of varying depths being shallow in some and deeper in others. In some instances only a split is visible externally but the disintegration of the tissues would have proceeded inside the stem leading to the formation of cavities whose extent could not be easily gauged from outside. In the older palms the stem is rendered weak by the disintegration of the tissues and many of them break when strong winds blow. Sometimes the disintegrated portions drop off early exposing a dark flat fibrous surface. In such cases the damage is relatively superficial and further progress of deterioration is slow. Such palms may not easily break during gales.

The dark colour of the cracks and fissures is due to the invasion of the tissues by various weakly parasitic fungi. *C. paradoxa* was the most common

isolate obtained from the affected tissues. This fungus produces large numbers of dark coloured spores which contribute to the colour of the invaded tissues. Sporophores of *Ganoderma lucidum*, *Lenzites* sp., and *Polystictus* sp. have also been found growing on the affected portions of the stem. Narasimhan (1934) has recorded the occurrence of *Dædalea* sp. on such palms in Mysore.

In order to find out if any of these fungi were responsible for the damage, they were first isolated from the damaged tissues and brought into pure culture. These were utilised for inoculating the stem of middle-aged palms (20 years old). In one series, the inoculations were made by placing bits of culture on the unwounded stem. Over these sterilised wet cotton-wool was laid and then alkathene sheets were tied round the stem enclosing the inocula. In a second series the stem was wounded prior to inoculation. Suitable controls were maintained. When the palms were examined after 10 weeks no change was noticed in the first series of inoculations and the controls except for the development of innumerable lenticel-like eruptions on the stem under the alkathene. But in the second series, cavities, about an inch in depth had formed and the tissues had disintegrated to a distance of about two inches above and below the point of inoculation. The growth of the particular fungus was observed in the affected tissues. The vascular bundles were found to be sticking out as darkened fibres. The extent of damage was more in those inoculated with *C. paradoxa*, *Lenzites* sp. and *Ganoderma lucidum* and less in the ones with *Polystictus* sp. The results led one to infer that the fungi found on the affected palms in nature do not infect through healthy surface but only through previously damaged or wounded portions. In nature the sunscorch-affected portions should function as places of entry for these organisms. Once having entered the stem these organisms which are always present in the arecanut growing tracts of the south, cause disintegration of the tissues leading to the development of cavities and depressions.

The location of the affected portion always between the western and southern side of the stem indicated that the prime factor responsible for the damage should be the lack of protection from the heat of the afternoon sun. The damage is invariably confined to the palms on the outskirts, whose stem is exposed to the afternoon sun. In thinly planted gardens, palms in the interior also exhibit such damage as adequate shade is lacking and exposure to the sun is more widespread. The temperature on the surface of the stem on the four sides of palms in the shaded and exposed portions of the garden were recorded daily during the hot weather. It is reported that in the afternoon the western and southern surfaces of the stem of palms in the

exposed and unprotected parts registered about 8° to 18° F. over the temperature on the northern and eastern surfaces of the same palms. Such differences were not observed in the forenoon or in the shaded areas (*Progress Report of the Scheme for the Investigation of Stem-Breaking Disease of Areca*, 1953–54). This rise of temperature is bound to have an adverse effect on the surface layers of cells of the tender portions resulting in their drying. Splits and cracks result when further growth takes place and the dried portions do not grow to keep pace with the rest of the stem. Various fungi enter through these cracks and infect the internal tissues. Thus protection of the stem, especially the tender portions from the hot afternoon sun, is called for as the initial measure to prevent this type of damage.

Methods of Protection.—Normally the site recommended by experienced growers for the location of areca gardens is in a valley with hillocks on the south and west, which will cut off the afternoon sun. Slopes of hills facing west or south are to be avoided. It may not always be possible to select such situations. As an alternative step it will be necessary to provide shade on the west and south by raising several rows of shade trees in advance of planting areca. The trees selected should be quick-growing, capable of attaining the same height as areca and should be evergreen. Tall-growing plants like coconut, jack, *Aini* (*Artocarpus hirsuta*), casuarina and other similar ones selected according to the suitability to the locality should be planted in several staggered rows. This is to be carried out while planning an areca garden. But in existing gardens which are in need of protection other palliative measures have to be thought of. It is customary in some gardens to tie leaf-sheaths of areca or banana round the tender portions of the stem to keep off the sun. This is a good practice which can be followed with advantage. Another method of protection against sunscorch adopted in orange gardens and rubber estates is to whitewash the exposed portions of the main stem with lime. This method has been reported to have been tried on areca using zinc-white and lime. Though these trials have been of short duration only zinc-white is stated to be promising (*Progress Report of the Scheme*, 1953–54).

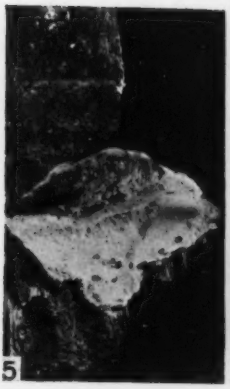
Raising mixed gardens of coconut and areca may prove beneficial in preventing sunscorch provided the areca palms are not on the outskirts. As a matter of fact splitting of the stem and stem-breaking are less common in such mixed gardens. In addition to the raising of shade trees on the outskirts it is necessary to maintain the interior of the garden in a cool and humid condition. Bananas are often grown intermixed with areca for this purpose. But the former is a heavy feeder and may be a keen competitor for areca in the removal of nutrients from the soil. Attention to proper

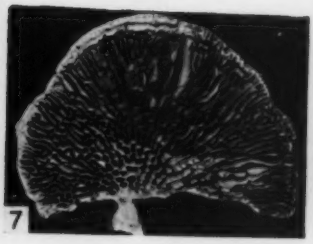
manuring with green leaves, potash, rock phosphate and well-preserved cattle manure will help to invigorate the palms and prevent their rapid deterioration. These should be worked into the soil or placed in a trench one-and-a-half to two feet away from the base and covered over and not dumped on the ground at the base as is being done in some gardens. The cultivation of cover crops like *Calopogonium mucunoides*, *Pueraria phaseoloides* or *Mimosa invisa* will be of great advantage as these will keep down the soil temperature and help to conserve soil moisture besides preventing soil erosion on hill slopes. The two latter plants have been tried with success in coconut gardens. To provide adequate supplies of green leaves, *Gliricidia maculata* may be grown along the borders and other vacant spaces. The loppings from these could be used for the supply of green leaves.

The palms which are in the initial stages of sunscorch damage, should be protected from further deterioration by removing all the affected tissues completely. The exposed surface should be protected by painting some fungicide like Bordeaux paste or Brunolinium. After the fungicide has dried a coating with some water-proofing substance like Stanvac Special Petrolatum should be applied to prevent water soaking in during the monsoon. These operations should be thorough to be useful. Partial or incomplete removal of the dead tissues will not prevent further damage. As a precautionary measure against the breaking of the stem, the damaged areca palms may be tied to neighbouring coconut palms or other tall and strong trees or among themselves by means of sufficiently long thick galvanised iron wire and thus prevent them from swaying too much in the wind. This will be similar to what is being adopted in parts of Travancore-Cochin State to prevent leaning coconut palms in the neighbourhood of buildings from falling over.

SUMMARY

The incidence of stem splitting and breaking is widespread in areca. This is not caused by the use of Bordeaux mixture for the control of fruit rot as was presumed by some growers. Exposure to the hot afternoon sun and the consequent scorching of the tender portions is the prime cause. Further damage is caused by the invasion of the affected tissues by more than one species of wound parasitic fungi. Provision of adequate shade is the best method of preventing this. The damaged portion is invariably between the western and southern sides of the stem. Any neglect in providing for shade at the planting time cannot be rectified easily later.





FIGS. 1–9

Literature Cited

1. Narasimhan, M. J. .. *Mysore Agric. Cal.*, 1934, 21–25.
2. Sundararaman, S., Krishnan Nair, C. K. and Ramakrishnan, T. S. *Bull. Indian Agric. Res. Inst. Pusa*, 1928, No. 189.
3. Venkatarayan, S. V. .. *Mysore Agric. J.*, 1931, 49–50.
4. Report .. *Progress Report of the Scheme for the Investigation of the Stem-Breaking Disease of Areca*, 1953–54.

Plate-Figs. 1–9. Figs. 1–3. Sunscorch effect on the stem (note the damage always on the same side). Fig. 4. A damaged palm longitudinally split during a gale. Fig. 5. Sporophore of *Ganoderma lucidum* (shown upside down). Fig. 6. Sporophores of *Polystictus* on the areca stem. Fig. 7. *Lenzites* sp. from areca. Fig. 8. Inoculated areca palm showing infection by *Ceratostomella paradoxa* in 10 weeks. Fig. 9. One method of protecting the tender stem by tying areca leaf-sheaths.

RED HALOPHILIC BACTERIA—THE IDENTITY OF SOME WELL-KNOWN SPECIES*

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(Communicated by Prof. P. S. Sarma, F.A.SC.)

The red halophilic bacteria from salts causing discolourations of salted fish and hides, have been studied for well over seven decades but till lately very little detailed account about their morphology, biochemical activities and taxonomy was available. Petter (1932), Lochhead (1934) and Gibbons (1936) described some of these bacteria in detail. Quite recently, Anderson (1954) and Venkataraman and Sreenivasan (1956) described a number of rod forms, mostly new species. Despite these researches, the position is still not satisfactory especially with regard to the coccus forms. For example, in the sixth edition of Bergey's Manual only *Sarcina littoralis* Poulsen is described, whereas in the literature numerous others are enumerated. In the Manual, *Micrococcus* (*Diplococcus*) *morrhuaæ* Klebahn is mentioned in Appendix III of the family *Micrococcaceæ*, as additional species, but in the proposed seventh edition of the Manual (in the press), *M. morrhuæ* is included in the genus *Micrococcus* and described in detail. *Sarcina morrhuæ* Farlow and *Micrococcus littoralis* Kellerman were considered synonymous, while *Diplococcus gadidarum* Beckwith and *M. littoralis gadidarum* were considered to be varieties of *S. littoralis* (Stuart, Frey and James, 1933). Lochhead classified Klebahn's *S. morrhuæ* and other similar organisms reviewed above as *Sarcina littoralis* Poulsen. Gibbons, in fact, preferred to designate this as *Sarcina* (*Micrococcus*) *littoralis*, since it was difficult to say whether the organism was a micrococcus or sarcina. In this confusion, the culture designated as *M. roseus halophilus* (Petrowa) or *Tetracoccus roseus halophilus* (Wlassowa) has escaped attention.

Methods

The cultures were studied by methods described in our earlier work (Venkataraman and Sreenivasan, 1956). The media used for growing them and for the study of physiological properties are described by Sreenivasan and Venkataraman (in press). Conventional media used by Gibbons (1936) were also tried. Tests for H_2S production were done according to Anderson's (1954) procedure.

* Published with the kind permission of the Addl. Director of Industries and Commerce, Madras.

Discussion

Four authentic coccus forms and four rod forms have been studied in detail and the results summarized in detail in Table I. It is clear that *S. littoralis*, *S. morrhuæ*, *M. morrhuæ* and *M. roseus halophilus* are four different species and not variants of a single species. Morphologically, *S. littoralis*, though described as a sarcina, does not show typical packets in all media or constantly in a single medium even. It is mostly seen as pairs or single or clusters even in wet mount preparations as well as in stained preparations. This is why probably Gibbons (1936) was inclined to call it a micrococcus, with which we also agree. On the other hand, *S. morrhuæ* always exhibits an arrangement in packets of three planes, consisting of 16, 32 or more cells and tetrads. In appearance while *S. littoralis* is smooth, *S. morrhuæ*, as it ages, shows rough, 'warty' dry folded brick-red appearance. Both are brilliant red in colour. *S. morrhuæ* slowly digests milk, produces alkaline reaction in sugars and also NH_3 from a salt-prawn broth-starch medium in contrast to *S. littoralis*. *M. morrhuæ* is orange red to bright red large coccus which does not show tetrad forms. This also digests casein slowly, and agrees with the description given in the seventh edition of the Manual. In our " Rice milk " medium, *S. morrhuæ* shows clearing of the medium due to digestion of casein and *M. morrhuæ* shows " Pits " or deep depressions due to starch digestion. *S. littoralis* and *M. roseus halophilus* grow on this medium without these changes. The latter is orange red to deep red as any of the above cultures. This is also a truly obligate halophile since it failed to grow even in 10% NaCl media. Though Petrowa (1935) had stated that this culture is a salt-adapted form of the ordinary mesophilic *M. roseus*, the culture studied by us could not be acclimatized to low-salt media. This culture was mostly in pairs, or short chains and sometimes in tetrads. On the other hand we had a truly rose-coloured culture isolated from salt-milk-agar tube as a (aerial ?) contaminant. This culture grew slowly and sparsely on 20% salt-milk-agar but grew also on sea-water agar and broth, while *M. roseus halophilus* failed to grow in liquid media. This latter culture was of further interest in producing indole. *M. rhodocrous*, a red coccus from sea-water, failed to grow in 20% salt-milk-agar though a slight growth was seen in 10% NaCl.

The following rod forms have been described well in literature—*Pseudomonas salinaria*, *Ps. cutirubra*, *Bacterium halobium* and *B. trapanicum*. In his excellent paper Lochhead (1934) had mentioned that *Ps. cutirubra* differs from *Ps. salinaria* in slight variation in colour and in its greater proteolytic action on milk. This itself is not sufficient to separate them but as seen from Table I there are differences warranting recognition of them as different species. Gelatin hydrolysis and nitrate reduction were positive in *Ps. cutirubra* but not

TABLE I

Morphological, cultural characteristics and physiological reactions of the red halophilic bacteria

Organism[1]	Cultural and morphological characters	Growth in 10% NaCl	Gelatin hydrolysis	Casein digestion	Nitrate reduction	Starch hydrolysis	Indole production	H_2S	Sugars
Pseudomonas salinaria	Circular, drop-like, entire, 2 mm. red. Deep red, smooth, filiform, moist growth in 2–3 days on salt-milk-agar. Very highly pleomorphic, motile rods. Simple rods rare; gram negative.	–	–	±	–	–	+	–	NC
Ps. cutirubra ..	Colonies as in *Ps. salinaria*. On salt-milk-agar, colour is darker, scarlet. On gelatin agar shows a a pink-mauve colour. Growth in less than 2 days. Highly pleomorphic, motile rods, more of simple rods with higher NaCl content; gram negative.	–	+ Beautiful pink-mauve colour	++	+	–	+	–	NC
Bacterium halobium ..	Circular, red, entire colonies on salt-milk-agar. Smooth, sometimes viscous growth, drawing out on wire. Good growth in 2–3 days on salt-milk-agar. Rods, long, thin, bent, rounded ends, gram negative, motile.	–	+ Mauve pink	±	–	–	+	–	NC

		bent, rounded ends, gram negative, motile.							
Bact. trapanicum	..	Rose-red, circular, entire; filiform, smooth, growth on salt-milk-agar in 4 days. Motile, simple straight rods, gram negative. Turbidity in broth also. Better growth on Rice milk medium	−	±	−	+	−	−	NC
Sarcina littoralis	..	Circular, convex, scarlet red, moist, glistening; smooth, butyrous, abundant in 2–3 days on salt-milk-agar. Also grows on Lochhead's yeast-starch agar. Cocci in pairs, single, clusters and tetrads; gram positive.	−	+	−	+	+	−	−	NC
Sarcina morrhuæ	..	Deep red, circular, entire, raised on salt-milk-agar; rough, dry growth on rice-milk-salt medium. Red, moist, butyrous, growth in 2–3 days on salt-milk-agar; on yeast-starch agar, also, dry "warty" rough growth characteristic. Cocci in packets of 16, 32, or more or tetrads ; gram positive.	−	+	+	+	±	−	−	Alk

TABLE I—(*Contd.*)

Organism[1]	Cultural and morphological characters	Growth in 10% NaCl	Gelatin hydrolysis	Casein digestion	Nitrate reduction	Starch hydrolysis	Indole production	H_2S	Sugars
Micrococcus morrhuæ	Orange-red circular, entire, raised, on salt-milk-agar. On rice-milk-salt medium, deep depressions characteristic; on starch media colour turns brick-red, dry. Large gram positive cocci, mostly pairs, single, rarely tetrads.	−	+	+	+	+	−	−	Alk (Sl)
Micrococcus roseus halophilus	Rose-red to deep red, circular, entire; smooth, moist, butyrous on salt. milk-agar in 2–3 days, cocci, gram positive, mostly pairs, a few tetrads and single.	−	±	−	±	−	+	−	Alk

− = Negative. ± = Partial or slow. NC = No change. Alk = Alkaline.

[1] Nomenclature : As furnished by the donors of cultures.

in *Ps. salinaria*. The former digests casein rapidly producing off-odours and shows beautiful change in colour from red to pink and mauve especially in gelatin media. With 25% NaCl more of simple rods are seen than pleomorphic forms. *Bacterium trapanicum* is of lighter rose-red shade in contrast to *B. halobium*, which also shows a mauve-pink colour in gelatin agar media. *B. trapanicum* is slower in growing than is *B. halobium* on salt-milk-agar. Further differences between the two are evident in the table. All the eight cultures are catalase positive.

Breed (1955) has proposed the creation of a new genus *Halobacterium* Elizari-volcani in family *Pseudomonadaceæ* for the polar flagellated, red brine organisms. The position of *B. halobium* and *B. trapanicum* whose flagellation is not clear, is not established but it is desirable to include them also in this new genus. The rod forms described by Anderson (1954) and Venkataraman and Sreenivasan (1956) also should belong to this genus. Though halophilic cocci and sarcina are not separated from their non-halophilic compeers it would be reasonable to accord them also a generic rank and designated as *Halococcus*, as suggested by Schoop (1934), in view of their obligate halophility.

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REFERENCES

Anderson, H. .. "The reddening of salted hides and fish," *Appl. Microbiol.*, 1954, **2**, 64.

Breed, R. S., Murrary, R. G. E. and Hitchens, A. P. *Bergey's Manual of Determinative Bacteriology*, 6th Edition, The Williams and Wilkins, Baltimore, U.S.A., 1948.

Breed, R. S. .. (Personal communication, 1955.)

Gibbons, N. E. .. "Studies on salt fish. I. Bacteria associated with the reddening of salt fish," *J. Biol. Bd. Can.*, 1936, **3**, 70.

Lochhead, A. G. .. "Bacteriological studies on the red discolouration of salted hides," *Canad. J. Res.*, 1934, **10**, 275.

Petter, H. F. M. .. "Over roode en andere Bakterien van gazouten Visch," *Thesis*, Utrecht, 1932.

Petrowa, E. K. .. "Etude sur le Pleomorphisme de L'Agent duRouge du Poisson salè," *Ann. Inst. Pasteur.*, 1935, **55**, 255.

Schoop, G. .. "Halophile Mikroben in Nahrungsmitteln Deutsch Tierärztl," *wochser.*, 1934, **42**, 816.

Sreenivasan, A. and Venkataraman, R. "Media for the study of red halophilic bacteria," 1956 (in Press).

Stuart, L. S., Frey, R. W. and James, L. H. "Microbiological studies of salt in relation to the reddening of salted hides," *U.S. Dept. Agric. Tech. Bull.*, 1933, **383**.

Venkataraman, R. and Sreenivasan, A. "Further studies on the red halophilic bacteria from salted fish and salts," *Proc. Ind. Acad. Sci.*, 1956, **45**.

561–56. Printed at the Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.

6

SPECTROCHEMICAL STUDIES ON THE UPTAKE OF IONS BY PLANTS

I. The Lundegårdh Flame Technique of Ash Analysis of Toxin/Antibiotic Invaded Cotton Plants

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Introduction

The Lundegårdh flame technique has been widely used for many investigations, as a flame emission spectrochemical tool for studying the distribution of many metallic ions in biological materials (Hultgren, 1932; Mitchell, 1948 and Lundegårdh, 1951). In this laboratory this technique has been standardised for the air-acetylene flame and used as a routine method in the investigation of ashes of both healthy and fungal root diseased plants, as also fungal ashes grown *in vitro* in synthetic nutrient substrate.

Using cut shoots of plants, for studies on toxin uptake and the consequent chelation with metallic ions, has been a standard method in most laboratories (Waggoner and Dimond, 1953; Gäumann and Naef-Roth, 1954, 1955 and 1956 and Gäumann, Naef-Roth and Kern, 1955). But in the results presented here, entire cotton plants grown in natural soils and soils infected with *Fusarium vasinfectum* Atk., have been studied instead of excised shoots. The importance of this investigation, hardly needs any favourable endorsement, as the mechanism of wilt produced by *vivotoxins* has remained obscure, despite much work on enzyme systems of the pathogens and also work on host physiology, especially on transpiration.

It was earlier indicated by one of us (Sadasivan, 1955), that toxins/antibiotics seem to interfere with enzyme systems and produced derangement of key metabolite(s). The present study of alkali and alkaline earth metals and iron and manganese and their distribution in healthy and diseased cotton plants was taken up to elucidate this postulate further.

Materials and Methods

Soil, pathogen and host.—Culture of *Fusarium vasinfectum* Atk., supplied by Centraalbureau voor Schimmelcultures (Baarn), was used to infect cotton plants, commonly called Karunganni 2 (*Gossypium arboreum*) grown in sterilised garden soil. Sterilisation of the soil and methods of artificial inoculation were as detailed previously (Sarojini, 1951).

Preparation of ash samples.—1 g. of oven-dry, finely ground plant samples were ashed for 8 to 10 hours at about 450° C., then taken to dryness twice with concentrated HCl on a steam-bath. This was then taken up in 5 to 10 ml. of 2 N HCl, filtered and made up to 50 ml. This formed the basic concentration for all analyses.

Standard solutions.—As is well known the Lundegårdh flame technique has the advantage, in that it carries duplicate spectrograms of the standard solution (Ca M/4,000; Sr M/10,000; Mg M/200; Na M/100; K M/200; Fe M/200; Mn M/4,000; and Li M/4,000) as well as its five dilutions, in N/20 HCl (Analytical reagent) on the same plate as neat, one-half, quarter, three-twentieths, one-tenth and one-twentieth and also spectrograms of unknown sample solutions.

The equipment.—A Hilger medium quartz spectrograph with Judd Lewis comparator and a non-recording microphotometer (mains operated and with a nickel/cadmium NIFE battery on floating circuit) formed the main equipment. The Lundegårdh flame was fed with acetylene at 20 ml. water manometer pressure and compressed air from an air compressor at 25 lb. per sq. in. A vent-axia fan at the end of a hood kept the flame steady (Mitchell, 1948). An A.C. spark between copper electrodes at 15,000 volts using ¼ K.W. transformer (F.S. 201 Hilger), ¼ K.W. condenser (F.S. 202 Hilger) and self-induction coil (F.S. 203 Hilger) and 40 ohms resistance was passed through the flame just above the blue cone (Hultgren, 1932). The self-inductance tapping was at 0·5 millihenries. This spark-in-flame increased the sensitivity of Mg, Fe and other alkali metals. Slit-width of 0·04 mm. was used throughout and the wedge adjusted to 1·5 mm. Both Ilford Zenith supersensitive and Ilford Q2 plates (10″×4″) were used for the photographs. The standard and the test solutions were sprayed from the Lundegårdh glass sprayer vessel as usual. Exposures were at 60 seconds throughout and the plates were developed at 20° C. for the recommended times in the proper developers. Photometry was done after spotting the lines on the comparator (Mitchell, 1948). The lines chosen for the elements under study were as follows:—K—4044/47 Å (as a doublet); Na—3302 Å; Mg—2852 Å; Ca—4226 Å; Mn—4030/33 Å (as a doublet) and Fe—2719 Å, (M.I.T. Tables). Although the most sensitive lines of K are the 7664/98 Å, the 4044/47 Å lines in the UV region were used as they were found to be quite satisfactory and obviated the use of Ilford Long Range spectrum plates for this element alone.

EXPERIMENTAL

Solutions of ash samples of 15-day old healthy and *F. vasinfectum* infected cotton plant shoots were carefully prepared as already detailed and

aliquots of these were sprayed into the flame (spark-in-flame). The spectra of the standard solution and its five dilutions preceded the spectra of the unknown samples. The solutions of the plant samples were further diluted for the determination of Ca, as the quanta of Ca present in the neat standard solution was much less than that occurring in 15-day old plant ash solutions of the basic concentration (*vide* Materials and Methods). The spectra were spotted for the lines of interest and read in the microphotometer. The transmissions of the lines in the spectra of the standard solutions, as seen by the galvanometric deflections was recorded and plotted to give the typical Lundegårdh working curve of the ratio of the galvanometer deflections of the line *plus* background, to background, plotted against concentrations. Similarly, the values of the lines of the unknown solutions were taken and juxtaposed on the standard curves and the quantitative estimations of the unknown samples arrived at. Table I summarises the results and gives the values for each element as p.p.m. or mg./g. dry weight.

TABLE I

The amount of K, Na, Mg, Ca, Mn and Fe in ash samples of 15*-day old cotton plants infected by* Fusarium vasinfectum

Results expressed as p.p.m. and mg./g. dry weight of plant tissue

	Dry weight in g.	Ash weight in mg.	K		Na		Mg		Ca		Mn		Fe	
			p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.
A ..	1·0	120·0	173·9	10·4	11·5	0·7	69·3	4·2	250·3	15·0	3·0	0·2	4·2	0·3
B ..	1·0	82·0	7·7	0·3	10·3	0·4	167·2	6·9	451·0	18·6	13·3	0·5	11·2	0·5

A—Healthy. B—Infected.

DISCUSSION

The critical period for the appearance of "vein clearing" symptoms in cotton seedlings from the time of germination, grown in *F. vasinfectum* infected soils has been variously stated as 10–15 days (Kalyanasundaram, 1954). The present set of infected and healthy plants were accordingly collected at the end of 15 days for ash analysis. It is obvious from Table I that K content in the infected plants goes down very considerably and, indeed, is most conspicuous possibly by virtue of its extreme mobility in the healthy host, where rapidity of entry is unhampered, whereas in the infected condition there obviously exists an osmotic barrier resulting in loss in permeability to a large extent to this particular element. The other alkali

metal Na shows very slight decreased accumulation in the infected over the control. On the other hand, the alkaline earth metals Mg and Ca behave dissimilar to the alkali metals inasmuch as they show increased quanta in the infected host tissues. The heavy metal Mn shows a very remarkable rise in quantity in the infected host tissue, a fact which is of considerable significance as it is known to chelate very readily with fusaric acid and might have been translocated as a chelate complex. Fe also registers an increase in the infected plant tissue, though not as spectacular as Mn. It is not clear, as to what significance is to be attached to the higher uptake of Fe and Mn by the infected plant, although it has to be stated that the Fe/Mn ratio of 4·2: 3·0 p.p.m. in the healthy is in the order of 11·2: 13·3 p.p.m. in the infected plant tissues. It may be that the uptake of essential K as also Mg and Ca have a bearing on the ratio of Fe/Mn. Earlier work in this laboratory (Varadarajan, 1953) had indicated that addition of Fe/Mn to *F. vasinfectum* infested soils in the ratio of 1 : 2 (40 p.p.m. Fe/80 p.p.m. Mn) did bring about a recovery on the 20th day from the wilt symptoms which were very prominent during the 13–14-day periods, after germination of the cotton plants.

It is not our intention at this stage to enter into any elaborate analysis of the causes of the wilt, but it may be pertinent to state that the present data clearly indicate a primary loss in permeability of the tissues with the onset of toxin invasion resulting in very considerable derangement (loss) of the entry of the key metabolite K and an accumulation of varying quantities of Mg, Ca, Fe and Mn. This deranged selective absorption or imbalance in ionic uptake as seen here, appears to lend less and less support to the vessel plugging theory (Scheffer and Walker, 1953) of Fusarial wilts, which may have to be discarded in the light of loss in cell functions in the root region and consequent deranged osmosis, now implied by this investigation.

SUMMARY

Spectrochemical analysis, using the standard Lundegårdh flame emission (spark-in-flame) method, of cotton plants infected by *Fusarium vasinfectum* Atk., showed an increased uptake of Mg, Ca, Fe and Mn with decreased accumulation of K over the healthy plants. It is suggested that the derangement in the selective absorption of ions, seen in the infected plants, is more likely to have been caused by chemical agents (toxins) than physical causes such as plugging of vessels.

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References

Gäumann, E. and Naef-Roth, St. — "Über die chelierende Wirkung einiger Welketoxine, I," *Phytopath, Z.*, 1954, **21**, 349–66.

——— .. "Über die chelierende Wirkung einiger Welketoxine, II. Die Verschiebungen der Toxizität durch steigende Zusätze von Asche aus jungen Tomatensprossen," *Ibid.*, 1955, **23**, 147–60.

——— .. "Über die chelierende Wirkung einiger Welketoxine, IV. Die Verschiebungen der Toxizität durch steigende Absättigung mit verschiedenen Schwermetallionen," *Ibid.*, 1956, **25**, 418–44.

——— Naef-Roth, St. and Kern, H. — "Über die chelierende Wirkung einiger Welketoxine, III. Die Verschiebungen der Toxizitat durch steigende Absättigung mit Eiseionen," *Ibid.*, 1955, **24**, 373–406.

Hultgren, R. .. "The spark-in-flame method of spectrographic analysis and a study of the mutual effects of elements on one another's emission," *J. Amer. chem. Soc.*, 1932, **54**, 2320–2328.

Kalyanasundaram, R. .. "Soil conditions and root diseases, XIII. Symptomatology of *Fusarium* wilt," *J. Indian bot. Soc.*, 1954, **33**, 330–37.

Lundegårdh, H. .. *Die Blattanalyse*, Hilger and Watts, Ltd., Hilger Division, London, England, 1951.

Mitchell, R. L. .. *The Spectrographic Analysis of Soils, Plants and Related Materials*, Commonwealth Bureau of Soil Science, Harpenden, England, 1948.

Sadasivan, T. S. .. "Symposium on soil micro-organisms and plant well-being," *Proc. Indian Acad. Sci.*, 1955, **41 B**, 99–101.

Sarojini, T. S. .. "Soil conditions and root diseases, II. *Fusarium udum* disease of red gam (*Cajanus cajan*)," *Ibid.*, 1951, **33 B**, 49–68.

Scheffer, R. P. and Walker, J. C. — "The physiology of *Fusarium* wilt of tomato," *Phytopathology*, 1953, **43**, 116–25.

Varadarajan, P. D. .. "Soil conditions and wilt of plants with special reference to trace element nutrition," *Doctoral Thesis*, University of Madras, 1953.

Waggoner, P. E. and Dimond, A. E. — "Role of chelation in causing and inhibiting the toxicity of Lycomarasmin," *Phytopathology*, 1953, **43**, 281–84.

ON *SPICULARIA TERRESTRIS* TIMONIN

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Spicularia terrestris was first described by Timonin in 1940, from an isolation from Canadian soils. Timonin's (1940, p. 314) description of the fungus was as follows: " Colonies on potato dextrose agar slow spreading, consisting mostly of submerged mycelium and aerial conidiophores; surface velvety in appearance, dark coffee-brown to fuscous (Ridgway, Plate XLVI); reverse black-brown. In rapidly growing colonies the hyphæ near the margin showing a yellow tint. Mycelium branched, sparsely septate with numerous terminal and intercalary dark olive-brown chlamydospores. Conidiophores arise from submerged or aerial mycelium, at the point of origin produces 1 to 2 rhizoid-like hyphæ; erect, slightly tapering towards apex, unbranched, septate, dark brown up to upper septum, smooth, up to 450 μ long. At the apex, which is slightly inflated, bearing a whorl (up to 12) of club-shaped sterigmata-like cells. Sterigmata are unbranched, non-septate and irregularly swollen at the apex covered with numerous papillæ. Sterigma are 16 to 18 μ long, 3 to 4·5 μ wide at the base, and the swollen part up to 8 μ thick. Conidia borne singly on each papilla, oblong-ovate, two-celled, slightly constricted at the septum; lower cell often bearing remains of papilla; light brown to fuscous, smooth, 6–9 × 3–5 μ ".

Spicularia Fuckel as typified by *S. icterus* Fuckel (Fuckel, 1870) belongs to the Dematiaceæ-amerosporæ and Timonin (1940) emended the generic diagnosis of *Spicularia* in order to accommodate his didymospored fungus in that genus. The validity of Timonin's emendment of Fuckel's genus (to the exclusion of its type, *S. icterus*) and his assignment of his fungus to *Spicularia* have rightly been questioned recently by Morris (1955) who found it necessary to keep Timonin's fungus and Fuckel's fungus in separate genera, since it had not been shown that Fuckel's fungus had didymospores and not amerospores. Indeed, type material of Fuckel's fungus is not available and, under the circumstances, Fuckel's description and figures have to be relied on entirely. It must be emphasised here that emendment of a genus to the exclusion of its type such as has been offered by Timonin (1940) is not warranted under the Rules. Accordingly, Morris (1955) proposed a new genus, *Umbellula* to accommodate *Spicularia terrestris* and named it *Umbellula terrestris* (Timonin) Morris. Morris (1955) characterised his new genus as follows: " Stalks of conidiophores simple, or sometimes

branched, especially in old cultures, erect, septate, dark, bearing at tip an umbelliform cluster of branchlets, each branchlet ending in a vesicular swelling on which conidia are borne singly on spicules, later proliferating at tip; conidia fuliginous, 1-septate". Morris placed his genus in the Dematiaceæ-Macronemeæ-Didymosporæ.

Recently, I reported this fungus from the Nilgiris (Southern India) as *Umbellula terrestris* (Subramanian, 1956).

However, the genus *Pseudobotrytis* Krzemieniewska and Badura recently described (Krzemieniewska and Badura, 1954) appears to antedate *Umbellula* Morris (1955) and would, therefore, have priority over the latter. Krzemieniewska and Badura's diagnosis of their new genus is as follows: "Conidiophores dark with transverse septa, on the top radially branched. The tips of the branchlets headlike swollen, covered with warts on which are seated dark, two-celled conidia. This genus belongs to the family *Dematiaceæ*, subfamily *Phæodidymæ*, group *Cordanea*, with one genus *Cordana*, from which it differs by the radial branching on the top of the conidiophore as well as by the way in which the conidia are attached. This last feature approached it to the genus *Botrytis* from which the name is derived" (Krzemieniewska and Badura, 1954, p. 774). Their description of the type species *P. fusca* K. and B. is as follows: "From the top of the dark-brown, in its upper part brighter coloured straight conidiophore (150–300 by 3·0 μ), which shows numerous septa, there start radially almost colourless branchlets 11·5–18·2 by 2·5–4·5 μ, ending in small heads that are 5·2 μ in diameter. On these heads covered with distinct warts are seated two-celled, dark-brown, spindle-shaped conidia 6·0–8·3 by 3·0–3·5 μ" (Krzemieniewska and Badura, 1954, p. 774).

A comparison of the description and figures of *Pseudobotrytis fusca* K. and B. and of *Umbellula terrestris* (Timonin) Morris (≡ *Spicularia terrestris* Timonin) shows that both the fungi are identical and congeneric. Since, of the two generic names, *Pseudobotrytis* and *Umbellula*, the former has priority and since Timonin's specific epithet appears to be the earliest applied to the fungus under discussion, I propose the following new combination:

Pseudobotrytis terrestris (Timonin) Subramanian comb. nov.

≡ *Spicularia terrestris* Timonin, 1940, *Canad. J. Res.*, C, **18**: 314.

≡ *Umbellula terrestris* (Timonin) Morris, 1955, *Mycologia*, **47**: 602.

= *Pseudobotrytis fusca* Krzemieniewska and Badura, 1954, *Acta Soc. bot. Poloniæ*, **23**: 774.

Umbellula Morris (1955) may accordingly be considered a synonym of *Pseudobotrytis* Krzemieniewska and Badura (1954).

SUMMARY

Spicularia terrestris Timonin is not congeneric with *S. icterus* Fuckel, the type species of *Spicularia* Fuckel. Morris (1955) proposed a new genus *Umbellula* to accommodate it and named it *Umbellula terrestris* (Timonin) Morris. However, the fungus is identical with *Pseudobotrytis fusca* K. and B. (the type species of the genus *Pseudobotrytis* K. and B.). Since *Pseudobotrytis* (1954) antedates *Umbellula* (1955) and since Timonin's specific epithet appears to be the earliest applied to the fungus, the new combination, *Pseudobotrytis terrestris* (Timonin) Subramanian is proposed. *Umbellula* Morris is reduced to synonymy with *Pseudobotrytis* K. and B.

REFERENCES

Fuckel, L. .. *Jahrb. Nas. Ver. Naturk.*, 1870, **23**, 359.

Krzemieniewska, H. and Badura L. " Przyczynek do znajomosci micro-organismow sciolki i gleby lasu bukowego. (A contribution to the knowledge of the micro-organisms from the litter and soil of beechwood)," *Acta Soc. bot. Poloniæ*, 1954, **23**, 727–81.

Morris, E. F. .. " A new genus of Dematiaceæ," *Mycologia*, 1955, **47**, 602–605.

Subramaniam, C. V. .. " Hyphomycetes—I," *J. Indian bot. Soc.*, 1956, **35**, 53–91.

Timonin, M. I. .. " The interaction of higher plants and soil micro-organisms—I. Microbial population of rhizosphere of seedlings of certain cultivated plants," *Canad. J. Res.*, 1940, **C 18**, 307–17.

CHROMOSOME STUDIES IN SOME INDIAN BARLEY—II

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Introduction

The problem of origin of agricultural strains of the common cereals has been dealt with recently by a number of workers. With the gradual invention of newer methods for a critical study of the karyotype, such investigations are being more and more facilitated. The details of the chromosome structure including even the minute differentiated segments can now be brought out at ease with the aid of the recent techniques.

Investigations have shown that the different strains of a single crop species may differ one from the other in their karyotypes, a difference which is detectable under the microscope. They suggest possibly that not merely gene mutations, but even visible structural changes of chromosomes may provide means for the origin of different agricultural strains. Such evidences have been gathered in case of *Hordeum vulgare* by Bhaduri and Sharma (1948), by Sharma and Bhattacharjee (1956) in species of *Sorghum*, etc. In these plants proof of the structural changes of chromosome possibly acting as a means in the evolution of strains, has been gathered. The present investigation with six different agricultural strains of *Hordeum vulgare* were undertaken with the same end in view, *i.e.*, to detect the role of structural changes of chromosome, if any, in their evolution.

MATERIALS AND METHODS

In the present investigation altogether six different strains of Barley (*Hordeum vulgare*) were taken into consideration. The different strains are as follows:

1. *Hordeum vulgare* .. Strain B 17
2. *H. vulgare* .. „ B 42
3. *H. vulgare* .. „ B 122
4. *H. vulgare* .. „ B 134
5. *H. vulgare* .. „ B 135
6. *H. vulgare* .. „ B 137

The above six different types had the grains markedly differing one from the other in external morphological details, *viz.*, size of the grains, shape, surface features (wrinkled or smooth, etc.), colour of endosperm, etc.

All these different strains of Barley seeds were obtained through the kind courtesy of the Head of the Division of Botany, Indian Agricultural Research Institute, New Delhi. These were all raised at the same Institution.

Method of germination

At first some (about 25–30) seeds of a strain were taken in a petri dish and the latter was properly labelled along with the strain number. A quantity of water was poured in the petri dish so as to plunge the seeds into water and thus to allow them to soak thoroughly. After about twenty-four hours the excess water was poured off from petri dish and the seeds were covered with a thin layer of saw dust and subsequently gently wetted by adding a few drops of water. In this way they were kept for another twenty-four hours, during which period the seeds germinated and from each individual came out a cluster of four to five roots (about 1–1·5 cm. long). The same method was followed for each and every strain investigated.

Fixation for somatic chromosomes

In order to study the somatic chromosomes and for better scattering and manifestation of morphological details, prior to fixation, the tissues were subjected to some pre-fixation chemical treatments.

The tip portions (about 3–4 mm.) of healthy roots were taken and were subsequently subjected to pre-fixation, fixation and staining procedure.

A number of techniques involving the use of Aesculine (Sharma and Sarkar, 1955), Coumarin (Sharma and Bal, 1953), Oxyquinoline (Tjio and Levan, 1950), Paradichlorobenzene (Sharma and Mookerjea, 1955), α-Bromo-

naphthalene and Oxyquinoline (Bhaduri and Ghosh, 1954), Chromic formalin, etc., were tried. Of these Paradichlorobenzene method yielded best results. The schedule in brief is as follows:

The healthy root-tips were treated in saturated aqueous solution of Paradichlorobenzene at 10–12° C. for three hours. These were then heated gently over a flame for three or four seconds in a mixture of 2% Aceto-orcein and N HCl mixed in the proportion of 9 : 1. The root-tip was then smeared in 1% Aceto-orcein by putting uniform pressure over the cover glass and blotting the excess stain.

The figures were drawn using a Leitz compensating eyepiece of × 20 and 1·3 apochromatic objective with a condenser 1·4 N.A. at a table magnification of approximately 3,000 times.

While drawing the idiograms the chromosomes have been arranged on the basis of their types irrespective of their sizes. That is why in some cases 'B' has been followed by 'A' and not *vice versa*.

Observations

Strain B 17.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 1, 1 *a*):

1. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

2. A pair of long chromosomes with nearly submedian primary constriction and a satellite at the end of the slightly longer arm (G).

3. Five pairs of medium-sized chromosomes with nearly median primary constriction (C).

Strain B 42.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 2, 2 *a*):

1. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

2. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

3. Four pairs of medium-sized chromosomes with median primary constriction in each (C).

4. A pair of short chromosomes with submedian primary constriction (D).

1
2
3
4
5
6
1a A B C C C C C
2a B A C C C C D
3a B C C C D D D
4a A F B C C C C
5a A F F B C C D
6a H H E A B C D

FIGS. 1–6

Strain B 122.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 3, 3 *a*):

1. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

2. Three pairs of medium-sized chromosomes with nearly median primary constrictions in two and nearly submedian primary constrictions in one (C).

3. Three pairs of short chromosomes with median primary constrictions (D).

Strain B 134.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 4, 4 *a*):

1. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

2. A pair of long chromosomes with submedian primary constriction (F).

3. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

4. Four pairs of medium-sized chromosomes with nearly median primary constrictions in three pairs and nearly submedian in one pair (C).

Strain B 135.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 5, 5 *a*):

1. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the shorter arm (A).

2. Two pairs of long chromosomes with submedian primary constrictions (F).

3. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

4. Two pairs of medium-sized chromosomes with median primary constrictions (C).

5. A pair of short chromosomes with submedian primary constriction (D).

Strain B 137.—The chromosomes of this strain could be classified into the following morphologically distinguishable types (Figs. 6, 6 *a*):

1. Two pairs of long chromosomes with median primary constriction (H).

2. One pair of long chromosomes with sharp median primary constriction and a secondary constriction very close to the primary one (E).

3. A pair of long chromosomes with submedian primary constriction and a satellite at the end of the comparatively shorter arm (A).

4. A pair of long chromosomes with median primary constriction and a satellite at the end of one of the arms (B).

5. A pair of medium-sized chromosomes with median primary constriction (C).

6. A pair of short chromosomes with submedian primary constriction (D).

DISCUSSION

The genus *Hordeum* has been considered by a number of cytologists as an attractive material for investigation and a good deal of work of importance have been carried out in the genus. As the members of this genus are regarded as important cereals, having much of economic importance, the significance of such studies from a practical standpoint is not negligible. Furthermore, it is fortunate that the chromosomes of *H. vulgare* are quite long and the diploid number is quite small, *i.e.*, $2n = 14$. These two facts are possibly responsible for their being the subject of attraction of a number of workers.

Chin (1941) reported the chromosome number of *Hordeum vulgare* as $2n = 14$, which has also been confirmed by a number of other investigators. His observations, mainly involving the number of chromosomes and satellites, led him to suggest a phylogeny of the species. While drawing up the ancestry of the species he has given considerable emphasis on deMol's concept of the nucleolar number as an index of polyploidy. It needs no mention that this concept in the light of the recent findings cannot account always for the increase in the number of nucleoli and other theories have been proposed providing evidences for additional means of such an increase. It has been suggested that non-homologous translocation involving nucleolar and non-nucleolar chromosomes may result in an increase in the number of nucleoli.

Recent investigations on a number of Indian strains of Barley (Bhaduri and Sharma, 1948) have revealed the presence of a high number of secondary constrictions in the different strains. Furthermore, some of the strains have been found to differ in karyotypes significantly from that of the others. The presence of supernumerary constrictions in some of the strains have also been recorded. On the basis of available evidences, emphasis has been laid on the role of structural changes of chromosomes in the evolution of different strains.

Their regular meiotic behaviour has been claimed as due to the homozygosity for translocation they have attained during evolution. The remarkably stable behaviour of the species is thus due to their homozygous constitution. Cross-breedings between different strains has been suggested as reliable methods for the mapping of such segmentally interchanged ends of chromosomes. This would be possible as ring formation is expected in such hybrids, at least in some of the crosses, which would lead to a detection of chromosome ends. It is worthy of note that recently Hagberg and Tjio (1952) have reported translocations in Barley. These two findings are no doubt significant.

The present report, which has dealt with the six different strains of Barley, indicate certain interesting peculiarities. Though all of them show chromosome number as $2n = 14$, karyotypic differences are noticed between different strains. This is apparent at a glance at their karyotypes. For example, the chromosome type " E " of " B 137 " where primary and secondary constrictions are very near each other could not be located in any of the other strains. Furthermore, there are six chromosomes with two constrictions in each, are to be found in this particular strain in contrast to others, where the number is less. The strain " B 122 " shows one pair of chromosomes with secondary constriction whereas the rest four strains are provided with four chromosomes with two constrictions. Amongst these rest four, the karyotypes reveal minor differences.

A significant fact emerging out from the above finding is relevant in connection with the problem of origin of different strains. It needs no emphasis that a study of their karyotypes, as the present findings reveal, may serve to some extent as a criterion for their identification. The evolution of these different varieties or strains, not necessarily involve imperceptible genic mutation, as is generally believed, but rather gross chromosomal changes have also taken place. At the present state of our knowledge, it is not possible to state precisely that such chromosomal rearrangements are responsible for their evolution. These facts are yet to be proved. This much can be said that if structual changes of chromosomes can have a reflection on the expression of the phenotype, which is an accepted fact, then the morphological or physiological differences of one strain from other can possibly be accounted for, on the basis of these changes.

In any case as all these varieties owe their origin to a common ancestor, either through direct or through indirect means, segmental homology of varying extent are expected between complements of different strains. In that case cross-breeding between themselves may give evidences of segmental

interchanges, manifested in their cytological behaviour. It is clear, however, that the homozygosity has been attained by these strains during evolution and judicial selection. If intercrosses are made and concrete evidences of segmental interchanges are obtained, mapping of chromosome ends might be possible in case of *Hordeum vulgare*. It is worthy of note that recent findings in this laboratory have also revealed such karyotypic differences between strains of *Sorghum vulgare* (Sharma and Bhattacharjee, 1956). More and more work in these lines in different cereals are desirable with the aid of the refined techniques.

SUMMARY

The present report has dealt with the study of the karyotypes of six agricultural strains of *Hordeum vulgare* raised at the Indian Agricultural Research Institute, New Delhi. The observations have been carried out following temporary squash preparations according to Paradichlorobenzene method suggested by Sharma and Mookerjea (1955). The types are:

1. *Hordeum vulgare* strain B 17
2. *H. vulgare* „ B 42
3. *H. vulgare* „ B 122
4. *H. vulgare* „ B 134
5. *H. vulgare* „ B 135
6. *H. vulgare* „ B 137

All of them possess fourteen chromosomes, but the karyotypes differ one from the other. The number of chromosomes with two constrictions have been found to be four in strains B 17, B 42 and B 134; six chromosomes with two constrictions each, occur in strain B 137. In addition, gross differences in karyotype have been noticed in a number of them. It has been suggested that the structural changes of chromosomes provide a means for the evolution of these strains.

REFERENCES

Bhaduri, P. N. and Sharma, A. K. "Karyotype analysis in some Indian strains of Barley," *Abs. Proc. 35th Ind. Sci. Congress*, 1948.

——— and Ghosh, P. N. .. "Chromosome squashes in cereals," *Stain Tech.*, 1954, **29**, 269–76.

Chin, T. C. .. "The cytology of some wild species of *Hordeum*," *Ann. Bot.*, 1941, **5**, 535–45.

Hagberg, A. and Tjio, J. H. .. "Cytogenetical studies on some Homozygous translocations in Barley," *Anal. Estac. Exp. de Aula Dei.*, 1952, **2** (3–4), 215–23.

Sharma, A. K. and Bal, A. K. .. "Coumarin in chromosome analysis," *Stain. Tech.*, 1953' **28,** 255–57.

——— and Mookerjea, A. .. "Use of Paradichlorobenzene in chromosome analysis," *Ibid.*, 1955, **29,** 1–7.

——— and Sarkar, S. .. "A new technique for the study of chromosomes of palms," *Nature*, 1955, **176,** 261–62.

——— and Bhattacharjee, Dipti " Cytogenetics of *Sorghum*," 1956 (In Press).

Tjio, J. H. and Levan, A. .. "The use of oxyquinoline in chromosome analysis," *Anal. Estac. Exp. de Aula Dei.*, 1950, **2** (1), 21–64.

Explanation of Figures

Figs. 1–6 *a*. Figs. 1 and 1 *a*. Somatic metaphase and idiogram of *Hordeum vulgare*, Strain No. B 17. Figs. 2 and 2 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 42. Figs. 3 and 3 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 122. Figs. 4 and 4 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 134. Figs. 5 and 5 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 135. Figs. 6 and 6 *a*. Somatic metaphase and idiogram of *H. vulgare*, Strain No. B 137.

OBSERVATIONS ON THE COMPOSITION OF BOTTOM MUDS IN RELATION TO THE PHOSPHATE CYCLE IN THE INSHORE WATERS OF THE MALABAR COAST*

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I. Introduction

As mentioned in a previous publication (Seshappa, 1953 *a*) observations were started in January 1952, on the bottom muds of the West Hill Sea near Calicut (Malabar Coast) with a view to elucidating their role in the inshore phosphate cycle, this latter being known to be of great importance in determining the biological cycles and fishery productivity of any aquatic area. The studies consisted of routine examination and analyses of mud samples from two selected stations (4 metres and 19 metres) for temperature, pH, percentage of silt colloidal matter, moisture percentage and also the interstitial and adsorbed phosphate. Total phosphorus was also determined on some occasional samples. Bottom water samples were collected simultaneously with the mud samples from both the stations and studied for temperature, pH, chlorinity and dissolved inorganic phosphate. The work was continued upto March 1953; the results obtained form the subject matter of the present paper and show that the muds are of great importance as reservoirs of phosphate.

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We are thankful to Dr. N. K. Panikkar for suggesting this work and for valuable discussions both during the progress of the work and in the preparation of the same for publication.

II. Methods

Collection of samples.—The mud samples were collected by means of a grab sampler and were taken from the surface 4″ of the sea bottom. Water samples were taken by means of a Casella bottle.

Temperature.—The temperature of mud was taken by inserting a thermometer deep in the centre of the grab sample as soon as the sample was taken aboard, the sample being as little disturbed as possible while taking it out of the grab.

Silt suspension.—A known weight of the mud (wet weight) was taken and a silt suspension was prepared after passing the sample through a set of sieves of the mesh types described by Rochford (1951). The suspension was made up to one litre and used for analysis.

Moisture content.—A known wet weight of the mud sample was taken and dried to constant weight at 105° C. in an electric hot-air oven. Three sub-samples were analysed for moisture content in each sample. The moisture content is expressed as a percentage of wet weight.

Silt percentage.—This was estimated as follows: The silt suspension was prepared as mentioned above. 50 c.c. of the suspension was taken before the samples for phosphate determination were taken and 50 c.c. afterwards. The two were dried to constant weight at 105° C. and these values used along with the dry weight and wet weight of a known quantity of mud, gave the silt content of the mud samples.

The analytical methods followed in this investigation were the same as described by Rochford (1951). pH determinations were made by shaking a small quantity of the mud with 20 ml. of distilled water, filtering and using cresol red indicator with the filtrate. A BDH Lovibond comparator was used for arriving at the pH values.

No preservative was used with the samples. They were all collected early in the morning and analysis was started as soon as they reached the laboratory, usually by about 10 A.M.

Water samples were also analysed fresh for pH and phosphate content. The water layer immediately above the bottom where mud samples were taken, was the one sampled for this purpose.

III. RESULTS

Temperature.—Tables I and II show the mean monthly values of the mud and water temperatures for both the stations.

TABLE I

Monthly average values of the temperature of the bottom mud and of the overlying water, in degrees centigrade in Station I

(4 Meters)

Month		No. of samples examined	Surface water	Bottom water	Mud	Remarks
January	1952 ..	5	29·3	28·9	29·2	
February	,, ..	4	30·1	30·0	29·9	
March	,, ..	4	30·4	30·3	30·5	
April	,, ..	4	31·6	31·3	31·2	
May	,, ..	3	30·7	30·6	30·9	
June	,, ..	..	..	..	..	
July	,, ..	2	26·1	24·7	25·5	Mud value from 3 samples
August	,, ..	4	25·4	23·9	24·8	
September	,, ..	5	25·9	23·9	23·7	
October	,, ..	3	27·2	26·1	26·2	
November	,, ..	3	28·5	28·3	28·4	
December	,, ..	5	28·4	28·2	29·5	Mud value from single sample
January	1953 ..	4	29·0	28·9	29·3	
February	,, ..	4	28·7	28·8	29·3	
March	,, ..	3	30·5	30·2	30·3	

The temperature of bottom water ranges between 23·9° C. and 31·3° C. while that of the bottom muds between 23·7° C. and 31·2° C. in Station I. The corresponding values for Station II are 21·9° C. to 29·7° C. and 22·1° C. to 29·6° C. respectively. In general, there is not much difference between the temperature of the muds and of the overlying water. The temperature is very high between March and May, the highest recorded value being in

TABLE II

Monthly average values of the temperature of the bottom mud and of the overlying water, in degrees centigrade in Station II

(19 Metres)

Month	No. of samples examined	Surface water	Bottom water	Mud
December 1951 ..	1	27·9	27·8	28·9
January 1952 ..	5	28·0	28·1	28·1
February ,, ..	4	28·8	28·8	28·9
March ,, ..	4	29·7	29·7	29·3
April ,, ..	4	30·0	29·7	29·6
May ,, ..	3	29·8	28·9	29·0
June ,, ..	..	..	..	..
July ,, ..	2	26·6	22·3	22·4
August ,, ..	4	24·6	21·9	22·1
September ,, ..	5	24·8	22·4	22·4
October ,, ..	3	25·7	24·0	24·2
November ,, ..	3	27·3	26·7	26·4
December ,, ..	1	28·0	27·8	27·6
January 1953 ..	4	28·0	27·8	27·5
February ,, ..	4	28·2	28·2	28·2
March ,, ..	3	28·9	28·7	28·4

the month of April. Lowest temperatures are observed during the active period of the south-west monsoon. Thus the annual temperature range for bottom water as well as for the muds is about 7·5° C. On a closer examination, it is seen that during the period when the monsoon is active, the differences in temperature between the bottom water and muds are slightly higher than at other times, the muds being somewhat warmer than the overlying water. This is marked particularly in Station I.

Hydrogen-ion concentration.—The pH values of bottom water (Table III) vary between 7·6 and 8·7 and those of muds between 7·6 and 8·1 in Station I

and the pH ranges for the water and the muds in Station II are 7·9–8·8 (?) and 7·4–8·1 respectively.

TABLE III

Monthly average values of pH for the mud and bottom water in Stations I and II of the West Hill Sea

Month		Station I			Station II		
		No. of samples examined	Water	Mud	No. of samples examined	Water	Mud
January	1952 ..	5	8·7	8·1	5	8·8 (?)	8·3
February	„ ..	4	8·6	8·1	4	8·7	8·1
March	„ ..	4	8·6	7·7	4	8·7	7·6
April	„ ..	4	8·4	7·6	4	8·6	7·5
May	„ ..	3	8·2	7·6	3	8·4	7·9
June	„ ..	2	8·2	7·7	2	8·3	7·4
July	„ ..	2	8·0	7·9	3	8·0	7·7
August	„ ..	4	7·8	7·7	4	8·1	8·2
September	„ ..	5	7·6	7·9	5	7·9	7·8
October	„ ..	3	8·3	8·1	3	8·4	8·3
November	„ ..	3	8·3	8·1	3	8·4	8·2
December	„ ..	5	8·6	..	..	..	..
January	1953 ..	4	8·4	8·0	4	8·5	7·9
February	„ ..	4	8·5	8·0	4	8·6	7·9
March	„ ..	3	8·5	8·0	3	8·6	8·0

It may thus be seen that there are marked differences in pH values of the muds and the water immediately above. Low pH values are seen between March and September and to a lesser extent in January and February. The pH of the muds is lowest during the monsoon months. The pH values of the water column immediately above the muds also show the same trends, but unlike in the case of muds, the pH does not remain low for a long time.

Chlorinity of the bottom water.—The bottom water does not show much asonal fluctuation in the chlorine content.

TABLE IV

Monthly average values of the chlorinity of bottom water at Stations I and II of the West Hill Sea

Month		No. of samples analysed	Station I	Station II
March	1952	4	19·19	19·06
April	„	4	19·28	19·16
May	„	3	19·35	19·43
June	„	2	19·54	19·19
July	„	3	18·53	19·25
August	„	4	18·35	19·09
September	„	4	19·02	18·90
October	„	3	18·86	18·96
November	„	2	19·36	19·26
December	„	1	18·72	19·28
January	1953	4	17·74	17·84
February	„	4	18·24	18·25
March	„	1	18·74	18·85

The mean annual range both for Stations I and II is about 1·6‰ to 1·8‰. The mean values actually vary between 17·74‰ and 19·54‰ in Station I and between 17·84‰ and 19·43‰ at Station II. Thus compared to the seasonal variations in the surface chlorinity (George, 1954 and Seshappa, 1953 *b*), the variations in the chlorinity of the bottom waters are rather low. The effect of these variations in chlorinity on the character of the bottom muds appears to be negligible. It is only when the chlorine content varies over wide limits as in an estuarine environment from almost fresh-water to typically marine conditions that the character and consistency of the muds are affected.

Nature of the muds.—The percentages of moisture and silt colloidal matter in the muds collected in the different months is given in Table V.

TABLE V

Average monthly values of moisture and silt percentages in mud samples from the Stations I and II of the West Hill Sea

Month	Station I			Station II		
	No. of samples analysed	Moisture %	Silt %	No. of samples analysed	Moisture %	Silt %
December 1951 ..	1	72·1	85·9	1	63·5	80·0
January 1952 ..	5	73·4	94·4	5	67·5	86·5
February „ ..	4	71·9	94·6	4	64·4	91·4
March „ ..	3	70·1	94·5	4	65·7	95·1
April „ ..	4	69·8	92·4	4	64·8	92·4
May „ ..	3	68·1	89·6	3	71·7	95·4
June „ ..	2	63·4	84·9	2	53·1	83·6
July „ ..	2	65·3	86·9	3	56·5	81·6
August „ ..	4	69·1	86·3	4	60·8	89·5
September „ ..	5	71·5	94·1	5	63·0	92·5
October „ ..	3	73·1	95·4	3	60·1	89·1
November „ ..	3	69·5	95·4	3	63·7	95·7
December „ ..	..	..	..	1	67·4	93·9
January 1953 ..	2	72·3	94·6	2	63·0	87·9
February „ ..	4	70·2	93·4	4	62·3	89·6
March „ ..	2	70·3	96·2	2	66·9	90·0

In Station I, the average moisture content of the mud varies between 63·4% and 73·4% whereas in Station II it ranges between 53·1% and 71·7%. The moisture values for Station II are lower than those for Station I except during May 1952. It may be seen that the moisture content is lowest during the monsoon period and highest during the relatively calm non-mon-

soon months. The variations in silt colloidal matter like those of the moisture content have shown a definite seasonal trend. Low values for silt content are observed in general during the monsoon months and to a lesser extent in December and January. Due to intense agitation or churning of the entire water column and, with it, the muds, the finer particles are taken up by the water and transported or " broadcast " over a much wider area. The result of this is a reduction in the silt percentage of the bottom muds. It is well known that the moisture-holding capacity of sediments increases with reduction in the particle size and *vice versa* (Sverdrup, Johnson and Fleming, 1942, p. 994). Figure 1 shows clearly the correlation between moisture and silt content of the muds in both Stations I and II. During the monsoon, the mud is composed of relatively coarse particles and hence has a low moisture content. These variations in the consistency of the muds influence to a large extent the release of the inorganic nutrients to the productive zone.

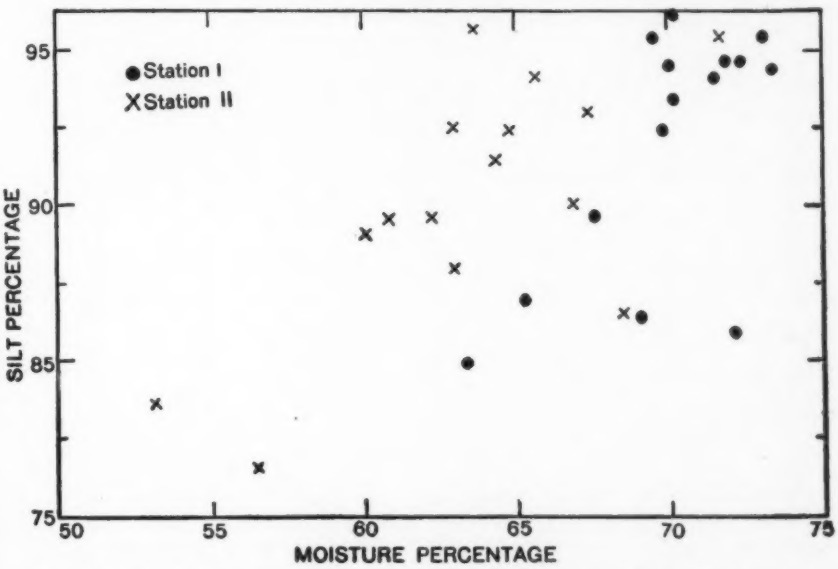

FIG. 1. Correlation between Moisture Content and Silt Content from Stations I and II.

Phosphate

Interstitial and adsorbed phosphates.—The interstitial and adsorbed phosphate values for Stations I and II are shown in Table VI and the seasonal trends are clearly seen in Figs. 2 and 3.

TABLE VI

Monthly average values of interstitial and adsorbed phosphates and also the interstitial phosphate/adsorbed phosphate ratios for the muds from Stations I and II of the West Hill Sea

Month	No. of samples analysed	Station I			Station II		
		Interstitial P. μg. PO_4-P/g.	Adsorbed P. μg. PO_4-P/g.	Interstitial P./ Adsorbed P. ratio	Interstitial P. μg. PO_4-P/g.	Adsorbed P. μg. PO_4-P/g.	Interestial P./ Adsorbed P. ratio
January 1952 ..	5	26	47	0·55	25	33	0·76
February ,, ..	4	35	21	1·67	29	16	1·82
March ,, ..	3 (Station I) 4 (Station II)	23	25	0·92	20	24	0·83
April ,, ..	4	17	24	0·71	15	18	0·89
May ,, ..	3	15	27	0·55	33	14	2·36
June ,, ..	2	13	23	0·61	9	10	0·90
July ,, ..	3	15	28	0·54	10	15	0·67
August ,, ..	4	15	40	0·38	16	9	1·78
September ,, ..	5	20	31	0·65	17	8	2·78
October ,, ..	3	22	39	0·56	19	6	3·17
November ,, ..	3	23	32	0·72	20	7	2·86
December ,, ..	1 (Station II only)	..	..	..	24	11	2·18
January 1953 ..	2	14	33	0·42	19	3	6·33
Februray ,, ..	4	19	46	0·41	17	14	1·21
March ,, ..	2	23	40	0·58	17	14	1·21

It may be seen that the lowest values for the interstitial and adsorbed phosphates occur during the monsoon period in the two areas. The seasonal trend is much better represented in Station I than in Station II. April–August is the period of lowest interstitial phosphate in the muds for Station I while for Station II it is April–September with an increase in May. The adsorbed

phosphate content is much lower in Station II as compared with Station I and also very low values persist for a longer period in the former area. The ratio of interstitial phosphate to adsorbed phosphate is, in general, much higher in the more offshore 19 metre area (Station II).

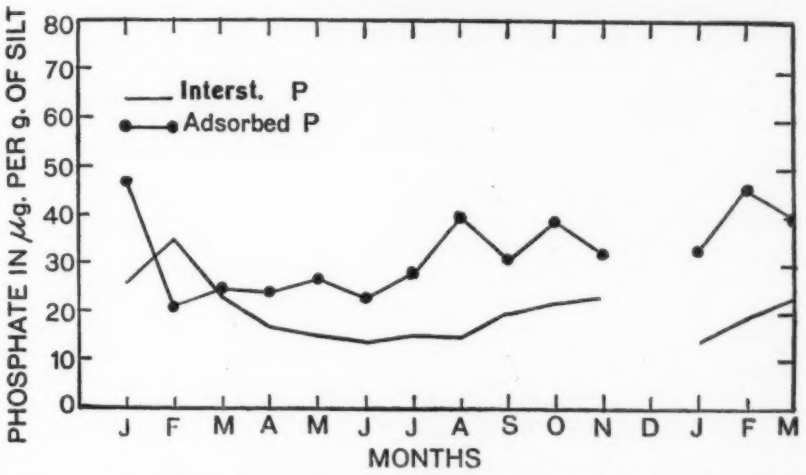

FIG. 2. Monthly Mean Values of Interstitial and Adsorbed Phosphate in the Mud from Station I during the Period January 1952 to March 1953.

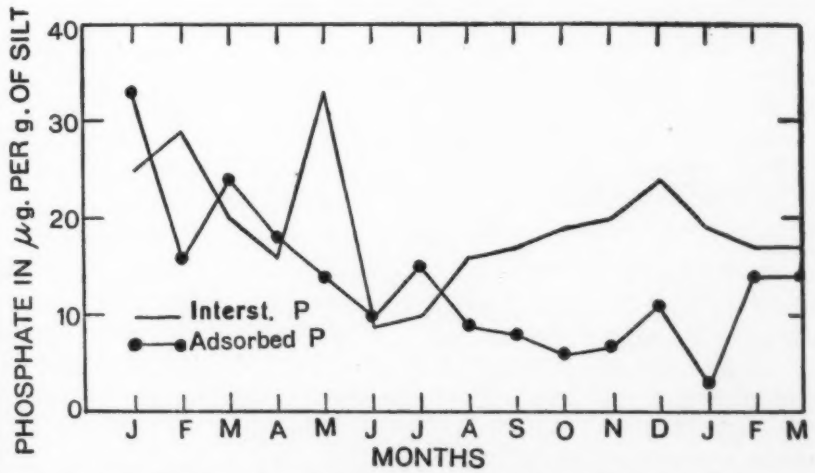

FIG. 3. Monthly Mean Values of Interstitial and Adsorbed Phosphates in the Mud from Station II during the Period January 1952 to March 1953.

Total phosphorus.—The total phosphorus content of the muds (combined organic and inorganic) of the two areas in the various months are given in Table VII.

TABLE VII

Average monthly values of total phosphorus in the muds of Stations I and II of the West Hill Sea

Month		No. of samples examined	Total Phosphorus (μg. PO_4-P/g.)	
			Station I	Station II
January	1952	1	2,500	1,200
April	,,	1	1,500	600
July	,,	1	1,650	1,210
August	,,	3	2,078	1,535
September	,,	2	1,355	920
October	,,	3	1,300	1,173
November	,,	3	1,150	1,260
December	,,	1 (Station II only)	..	1,280
January	1953	2	1,508	1,075
February	,,	3	1,629	1,662
March	,,	2	1,349	1,538

The muds in the inshore area (Station I) are much richer in the phosphorus content than those of the more offshore region (Station II). The mean monthly values for the inshore muds range between 1,150 and 2,500 μg./g. while those for Station II fluctuate between 600 and 1,662 μg./g.

IV. DISCUSSION

Data relating to the various phosphate fractions in the muds of Stations I and II (Table VI) show that the maximum release of phosphates takes place during the monsoon months. Miller (1952) working on the bottom deposits of Biscayne Key, Florida, found that there was an enrichment of the water phosphate regularly every summer from the bottom muds very much as in the present case. He also observed that the transfer of phosphate from sediment to water was accompanied by a slight decrease in pH. It has been found in the present investigations that during the period when the interstitial phosphate values for the muds are low, the pH values are also low (Fig. 4).

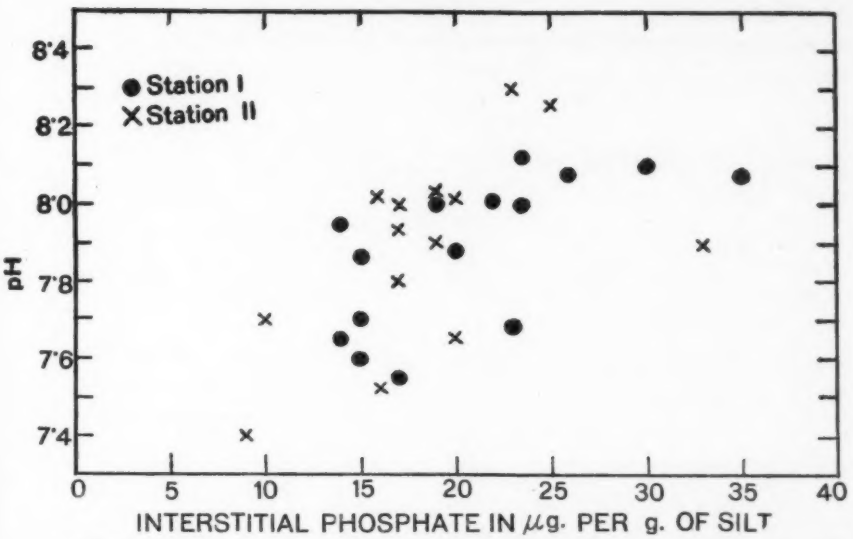

FIG. 4. Correlation between pH and Interstitial Phosphate of Mud Samples from Stations I and II.

The interstitial phosphates are, in general, very low during the period of the monsoon and the reduction in this phosphate fraction is due to the leaching effect on the immediate surface layers. The degree of leaching is, to a large extent, dependent on the *permeability* of the mud surface. The permeability of the mud in turn, is determined by its porosity, particle size and " compaction " and is a measure of the ability of the water to circulate through it (Sverdrup *et al.*, *loc. cit.*, p. 933). As stated earlier, the intense agitation or churning during the monsoon helps to transfer the phosphates from the interstices of the mud to the overlying water. It could also be seen that while there is reduction in the values for interstitial phosphates, the phosphate content of the bottom water in both the stations increases considerably during the monsoon (Table VIII).

The adsorbed phosphate shows reduction in concentration similar to the interstitial phosphate during the monsoon period. According to Rochford (1951, p. 37) there appears to be an equilibrium between the interstitial and adsorbed forms of phosphate. So with the progressive removal of interstitial phosphate more and more of adsorbed phosphate should be brought into the interstices and thence to the water column. During the non-monsoon months the permeability of the muds is very low in view of the high silt content

TABLE VIII

Average monthly values of inorganic phosphate content in the bottom water at Stations I and II of West Hill Sea

Month				μg. PO_4-P/L of water	
				Station I (4 Metres)	Station II (19 Metres)
January	1952	..	..	10	7
February	,,	..	..	13	8
March	,,	..	..	12	10
April	,,	..	..	9	8
May	,,	..	..	9	10
June	,,	..	..	12	12
July	,,	..	..	53	61
August	,,	..	..	101	83
September	,,	..	..	107	58
October	,,	..	..	63	56
November	,,	..	..	22	22
December	,,	..	..	23	10
January	1953	..	..	11	5
February	,,	..	..	11	8
March	,,	..	..	15	11

and maximum " compaction ". This results in the retention of large quantities of phosphates in the interstices of the muds while the overlying water has low concentration of phosphates.

One of the possible sources of phosphate in the bottom muds off the Malabar Coast as stated by Seshappa (1953 *a*) is the run-off during the monsoon months bringing with it fine particles of laterite containing a lot of adsorbed phosphate. The sources of phosphate regeneration in the local turnover are also of great importance though the details relating to this aspect will not be discussed here. An important factor affecting the local turnover in the West Hill Sea must, however, be mentioned, namely, a large-scale mortality of bottom animals in the inshore regions at the commencement of

the monsoon (Seshappa, 1953 *b*). Such a mortality must naturally result in a chain of events leading to a considerable increase in the phosphates of the bottom deposits the bacteria playing a dominant role in these changes (Stephenson, 1949; Barnes, 1954; Harvey, 1955).

V. Summary

Data have been collected on the temperature, pH, silt colloidal matter, moisture and phosphate content of mud samples from two selected stations of the West Hill Sea along the Malabar Coast.

The variations in the several factors have revealed a distinct seasonal trend, the values being, in general, low for all the factors during the monsoon months.

The bottom muds contain large quantities of interstitial and adsorbed phosphates in the non-monsoon period. During the monsoon there is a rapid release of phosphates into the overlying column of water.

The factors associated with the monsoon release of phosphates from the muds are examined.

VI. References

Barnes, H. .. *Chemical Aspects of Oceanography—Lectures, Monographs and Reports, No.* 4. The Royal Institute of Chemistry, London, 1955.

George, P. C. .. "The Marine Plankton of the Coastal waters of Calicut with observations on the Hydrological conditions," *J. Zool. Soc. India*, 1953, **5** (1), 76–107.

Miller, S. M. .. "Phosphorus exchange in a sub-tropical Marine Basin," *Bull. Marine Sci. Gulf. and Caribbean*, 1952, **1** (4), 257–65.

Harvey, H. W. .. *The Chemistry and Fertility of Sea Waters*. Cambridge University Press, 1955.

Rochford, D. J. .. "Studies in Australian estuarine hydrology. I. Introductory and comparative features," *Austr. J. Mar.* and *Freshw. Res.*, 1951, **2** (1), 1–116.

Seshappa, G. .. "Phosphate content of mud banks along the Malabar Coast," *Nature*, London, 1953 *a*, **171**, 526.

———— .. "Observations on the physical and biological features of the inshore sea bottom along the Malabar Coast," *Proc. Nat. Inst. Sci. India*, 1953 *b*, **19** (2), 257–79.

Stephenson, W. .. "Certain effects of agitation upon the release of phosphate from the mud," *Jour. Mar. Biol. Assoc.*, 1949, **28** (2), 371–80.

Sverdrup, H. U., Johnson, M. W. and Fleming, R. H. "Marine Sediments," *The Oceans—Their Physics, Chemistry and General Biology*, New York, 1942, pp. 946–1049.

ROLE OF TRACE ELEMENT CHELATION IN THE FUSARIUM WILT OF COTTON

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Introduction

The important role of chelation of phytotoxins with heavy metals *in vivo* and *in vitro* in the mechanism of fusariose wilts of plants has been emphasized by many workers (Gäumann, 1951; Dimond and Waggoner, 1953; Waggoner and Dimond, 1953; Lakshminarayanan, 1955 *a*, *b*). Lycomarasmin, the important toxic peptide synthesized by *Fusarium lycopersici* Sacc., has been known to be 10 times more toxic to cut shoots of tomato as its Fe‴ chelate than alone (Gäumann, 1951). Fusaric acid, a non-specific toxic metabolite synthesized by many Fusaria, has recently been established to be a vivotoxin in the Fusarium wilt of cotton and identified as its Cu″ chelate (Lakshminarayanan and Subramanian, 1955; Kalyanasundaram and Venkata Ram, 1956). The active toxic principle(s) in dialysed culture filtrate of *F. vasinfectum* was reported to be potentiated by Fe‴ and the mechanism of wilt resistance in cotton was explained to be the removal of free Fe‴ by cystine present in the resistant varieties of cotton through preferential chelation (Lakshminarayanan, 1955 *b*). Chelating agents thus offer a facile method of wilt control through the removal of free trace elements in susceptible hosts. A number of chelating agents—8-Hydroxyquinoline, Ethylene diamine tetracetate (EDTA), Cystine, Sequestrene compounds of heavy metals, common phyto-hormones, etc., have been screened for chemotherapeutic activity in the Fusarium wilt of cotton and preliminary results with 8-quinolinol *in vitro* and *in vivo* are presented herein.

Materials and Methods

Cut shoots of susceptible cotton plants (Karunganni 2-*Gossypium arboreum*) 10–12 days old were pretreated to the following concentrations of 8-quinolinol at room temperature for 24 hours before transferring to the culture filtrate of a pathogenic strain of *F. vasinfectum* (grown in Richard's medium 1·5 l. in 4 l. Haffkine flasks for 6 weeks, dialysed against distilled water for 72 hours at 4–5° C. and Seitz filtered) and observed for 48 hours both in daylight and in U.V. 3,560 Å (Subba Rao, 1954)—1·0, 5·0, 10·0, 20·0 and 100·0 p.p.m. maintaining a complete set of controls. In the next

series of experiments K 2 plants were treated to the above concentrations of 8-quinolinol in sand culture. The plants were grown in glazed earthenware pots (4 × 10″) containing 1 kg. of acid washed sand to which 560 ml. of Crone's medium were added and sterilized. The experimental series contained the various concentrations of 8-quinolinol in the total nutrient medium added. Five seeds were sown in each pot and the pots were weighed daily and sterile distilled water was added to make up the transpirational loss. The plants were grown for 10 days, carefully removed from the sand, roots washed well with sterile distilled water and inoculated by soaking in a heavy spore suspension of *F. vasinfectum* for 10 minutes after injuring them by pruning, taking the usual precautions to avoid contamination. The plants were transplanted into a new set of pots containing fresh sand and nutrient solution. The plants were observed for a period of 15–18 days when typical wilt symptoms appeared in the controls.

TABLE I

In vitro *Action of* 8-*Hydroxyquinoline on Cut Shoots of Cotton*

No.	Pre-treated				Not pre-treated			
	Daylight		Ultra-Violet		Daylight		Ultra-Violet	
	Tube I	Tube II	Tube I	Tube II	Tube I	Tube II	Tube I	Tube II
A	2·0	2·0	2·0	2·0	2·0	2·0	2·0	2·0
	3·0	3·0	3·0	3·0	3·0	D	3·0	D
B	1·0	1·5	1·0	1·0	2·0	2·0	2·0	2·0
	3·0	3·0	2·0	2·5	3·0	3·0	3·0	3·0
C	0·5	0·0	1·0	1·0	2·0	2·0	2·0	2·0
	1·0	0·5	1·0	1·0	2·0	3·0	2·0	2·0
D	2·0	2·0	1·0	2·0	2·5	3·0	2·5	3·0
	3·0	3·0	3·0	3·0	3·0	D	D	3·0*
E	2·0*	2·0*	2·0	2·0	2·0*	2·0*	3·0	3·0
	3·0*	3·0*	3·0	3·0	D	3·0*	D	3·0*

TABLE I—(*Continued*)

No.	Pre-treated				Not pre-treated			
	Daylight		Ultra-Violet		Daylight		Ultra-Violet	
	Tube I	Tube II	Tube I	Tube II	Tube I	Tube II	Tube I	Tube II
BL I	0·0	0·0	0·0	0·0				
	0·0	0·0	0·0	0·0				
BL II	2·0	2·0	2·0	2·0				
	3·0	3·0	3·0	3·0				
A′	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
	1·0	1·0	0·5	1·0	2·0	2·0	2·0	2·0
B′	0·0	0·0	0·0	0·0	0·0	0·0	0·0	0·0
	1·0	1·0	1·0	1·0	2·0	2·0	2·0	2·0
C′	0·0	0·0	1·0	1·0	0·0	0·0	1·0	1·0
	1·0	1·0	2·0	2·0	3·0	2·0	2·0	2·0
D′	0·0	0·0	1·0	1·0	0·0	1·0*	1·0*	2·0
	2·0	2·0	2·0	2·0	3·0	3·0*	3·0	3·0
E′	1·0	1·0*	2·0	2·0	1·0	1·0*	2·0	2·0
	2·0	2·0	3·0	3·0	3·0	3·0	3·0	3·0

A, B, C, D and E represent the various concentrations of 8-hydroxyquinoline: 1·0, 5·0, 10·0, 20·0, and 100·0 p.p.m. respectively.

A′, B′, C′, D′ and E′ represent corresponding controls without culture filtrate. BL I. Water blank. BL II represents controls with only 50·0% culture filtrate.

Daylight	Ultra-Violet
0·0 Completely healthy as Water blank.	0·0 Absence of fluorescence as Water blank.
1·0 Slight shrivelling, yellowing and vein-clearing symptoms on leaves.	1·0 Fluorescence of the stem.
2·0 Advanced foliar symptoms with petioles drooping but stem healthy.	2·0 Advanced fluorescence in the stem and petioles.
3·0 Complete stem necrosis with severe foliar injury.	3·0 Fluorescent symptoms along the veins on the dorsal side of leaves.

D in both cases represents complete death of plants.

* Severe stem necrosis.

The figures in the first line indicate the readings after 24 hours and those in the second line represent the readings after 48 hours.

TABLE II

Showing the Effect of 8-*Hydroxyquinoline in Sand Cultures*

No.	Treatment	No. of plants wilted			% Wilt
		Replicate I	Replicate II	Replicate III	
1	Con	Nil	Nil	Nil	0·0
2	Con_f	4	5	5	93·3
3	A	Nil	Nil	Nil	0·0
4	B	Nil	Nil	Nil	0·0
5	C	Nil	Nil	Nil	0·0
6	D	Nil	1	Nil	7·0 (Slightly toxic)
7	A_f	2	2	3	46·7
8	B_f	0	2	1	20·0
9	C_f	2	1	1	26·7
10	D_f	1	3	1	33·3

Con.: Normal healthy series.
Con_f: Inoculated series.
A, B, C, D: Represent in order 1·0, 5·0, 10·0 and 20·0 p.p.m. of 8-quinolinol.
A_f, B_f, C_f, D_f : Represent the inoculated series for the same concentrations of 8-quinolinol.
Each pot contained 5 plants and the figures represent the number of plants dead in each pot.
(E: 100·0 p.p.m. of hydroxyquinoline was highly toxic to the plants.)
Singnificance by 'F' Test: Yes.
Critical difference: (5%) level—21%.

Conclusion: $\overline{1\ 3\ 4\ 5\ 6\ 8\ 9\ 10\ 7\ 2}$·

RESULTS AND DISCUSSION

The results (Table I) indicated that 8-quinolinol while not toxic at concentrations 20·0 p.p.m. and below gave effective protection to the cut shoots against toxæmia at the optimum level of 5–10 p.p.m. However, cut shoots not pre-treated to 8-quinolinol but treated to mixtures of the culture filtrate and the chemotherapeutant to identical levels recorded typical wilt symptoms.

The results presented in Table II indicate that in the range of 5·0–10 p.p.m. 8-quinolinol confers effective protection to the susceptible plants. Concentrations higher than 10 p.p.m. though affording protection to an equal

extent to the host plants against wilt, produced toxic symptoms as stunting and dechlorophyllation. The statistical significance of the results is presented under Table II.

It would be quite evident from the foregoing results that the chemotherapeutic action of 8-quinolinol is possibly through the removal of free heavy metals in the host, thereby rendering them unavailable for chelation with the toxin, which seems to be a necessary prerequisite for toxicity. Since the chemotherapeutant is active only when the plants are pre-treated, it would be evident that the toxic metabolites have relatively higher chelating capacity than 8-quinolinol, while 8-quinolinol trace element complexes are more stable *in vivo*. It is also clear that these wilting toxins act not only as mere chelating agents causing the removal of essential heavy metals from the host tissues but require specific metals for chelation which renders the chelate toxic.

While the exact function of heavy metals in the synthesis or potentiation of vivotoxins is not clearly understood, there seems to be little doubt that they play a vital role in the manifestation of disease syndrome. As it has recently been indicated in this laboratory that an optimal concentration of zinc is essential for maximal synthesis of fusaric acid in pure cultures (Kalyanasundaram and Saraswathi-Devi, 1956) it could be logically concluded that optimal concentrations of heavy metals are essential for the synthesis of phytotoxins *in vivo* in the diseased hosts during pathogenesis. The important role of these trace elements as components of phytotoxic chelates has been emphasized earlier (*loc. cit.*). It is quite probable that 8-quinolinol functions as an effective chemotherapeutant in wilt control of cotton through preferential chelation to the available free trace elements in the host plants. The action of 8-quinolinol is thus comparable to that of cystine which has been reported to be one of the factors responsible for resistance to wilt in cotton. It is, therefore, the quantum of available free trace elements in the host plants that would determine susceptibility or resistance to wilt and the competition for chelation to the trace elements between the phytotoxins, host auxins and the chemotherapeutant and the relative stabilities of these *in vivo* during pathogenesis would largely determine the efficiency of the chemotherapeutant. The behaviour of 8-quinolinol in cotton wilt control appears to be analogous to its behaviour in the Fusarium wilt of tomato (Dimond, 1955). It looks improbable that phytotoxins, poor chelating agents as they are, in the concentrations they are formed *in vivo*, could disturb the intact trace elements in bound form (as protein complex, etc.), resulting in deficiency symptoms (Dimond, 1955). It is more reasonable to believe that the wilting toxins act not only by virtue of their chelating ability, but the *in vivo* chelation of these

with the heavy metals available in the host is a necessary prerequisite for the potentiation of vivotoxins during pathogenesis.

Work on the free and bound heavy metals in roots, shoots and leaves of susceptible and resistant varieties of cotton in the healthy and diseased conditions is underway and is likely to throw valuable light on the mechanism of heavy metal chelation in pathological wilting.

Summary

Pre-treatment to an optimum concentration of 5–10 p.p.m. of 8-quinolinol was found to give effective protection to cut shoots of cotton against toxæmia to dialysed culture filtrate of *Fusarium vasinfectum*. The chemotherapeutant was also effective *in vivo* in sand cultures. The possible role of 8-quinolinol in the cotton wilt control with special reference to heavy metal chelation is discussed.

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References

Dimond, A. E. .. "Pathogenesis in the wilt diseases," *Ann. Rev. Plant Physiol.*, 1955, **6**, 329.

——— and Waggoner, P. E. .. "On the nature of vivotoxins in plant disease," *Phytopathology*, 1953, **43**, 229.

Gäumann, E. .. "Some problems of pathological wilting in plants," *Advances in Enzymology*, 1951, **11**, 401.

Kalyanasundaram, R. and Saraswathi-Devi, L. "Zinc in the metabolism of *Fusarium vasinfectum* Atk.," *Nature*, Lond., 1956, **175**, 945.

——— and Venkata Ram, C. S. "Production and systemic translocation of fusaric acid in Fusarium infected cotton plants," *J. Indian bot. Soc.*, 1956, **25**, 7.

Lakshminarayanan, K. .. "Some biochemical aspects of vascular wilts," *Proc. Indian Acad. Sci.*, 1955 *a*, **41 B**, 132.

——————— .. "Role of cystine chelation in the mechanism of Fusarium wilt of cotton," *Experientia*, 1955 *b*, **11**, 388.

——— and Subramanian, D. .. "Is fusaric acid a vivotoxin ?" *Nature*, Lond., 1955, **176**, 697.

Subba Rao, N. S. .. "Fluorescence phenomenon in fusariose wilt of cotton," *J. Indian bot. Soc.*, 1954, **33**, 443.

Waggoner, P. E. and Dimond, A. E. "Role of chelation in causing and inhibiting the toxicity of lycomarasmine," *Phytopathology*, 1953, **43**, 281.

STUDIES IN THE GENUS PHYTOPHTHORA

III. Homothallic Strains of Phytophthora on *Areca catechu**

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It has been reported that Coleman (Narasimhan, 1930) observed oospores in a set of cultures of *Phytophthora palmivora* Butl. from areca wherein 'fresh aseptically taken arecanuts were placed in Roux tubes and inoculated with the mycelium of the fungus'. Rosenbaum (1917) has also reported the presence of oospores in the cultures of *P. palmivora* (*P. arecæ*) received from Coleman. Narasimhan (1930) has stated that he had examined many affected arecanuts and oats agar cultures of the fungus from areca but had never observed any oospores. Narasimhan (1930), Venkatarayan (1932) and Thomas and his co-workers (1947) were able to obtain oospores when isolates of *P. palmivora* from areca were paired with isolates from other hosts. Uppal and Desai (1939) however found that when two strains of the pathogen isolated from areca growing in two different regions were brought together oospores were formed. Thus they observed that both the complementary strains were pathogenic to areca. In all these studies the pathogen was found to be heterothallic, the two strains being parasitic on different hosts or on areca.

In recent years five new isolates of *P. palmivora* were obtained from diseased areca fruits from different parts of S. Kanara District. Detailed studies of these isolates have been made which revealed that areca is parasitised by more than two strains of the pathogen some of them producing oospores in pure cultures. The results of these investigations are communicated in this paper.

Materials and Methods

The isolates were brought into pure culture from single sporangia. The cultures were maintained at the laboratory temperature (26° to 29° C.). The media used in these studies were adjusted to pH 5·6. The pathogenic studies were conducted on healthy plants specially raised for the purpose. After inoculation the plants were covered by belljars or the inoculated parts were enclosed in alkathene bags. Some of the fruits like those of brinjal, tomatoes,

* The data included in this paper are from the thesis for the M.Sc. Degree, Madras University, of V. Seethalakshmi.

etc., were surface sterilised with 0·1 per cent. mercuric chloride solution and kept in sterilised moist chambers before inoculation. Five isolates were included in the studies. These are designated as A1, A2, A3, A4 and A5. The first two isolates were obtained from diseased areca fruits in 1951, from Mundaje (S. Kanara). The third and fourth were isolated from fruits collected from the neighbourhood of Vittal in 1952. The fifth isolate was made in 1953 from diseased specimens of fruits from Mangalore. To arrive at the mean dimensions of the different types of reproductive bodies, 200 individuals were measured in each, from cultures of the same age (20 days for asexual bodies and 25 days for sexual reproductive bodies).

Experimental Results

Cultural characters.—The nature of growth and reproduction of the five isolates on five media, *viz.*, oats, frenchbean, maize-meal, carrot and potato-dextrose agars, were observed.

Oats agar.—All the isolates produced fluffy white aerial mycelium. Sporangia were formed in 4 to 5 days. A1 formed numerous sporangia in a week while in the others the same intensity was reached in a fortnight only. Chlamydospores commenced to develop in all the isolates in 4 to 6 days, A1 being precocious. A1 and A2 did not form oospores but in the other three oospore formation was evident in 10 to 11 days and was abundant in 2 to 3 weeks.

Frenchbean agar.—All the isolates exhibited profuse cottony white aerial growth. Sporangia and chlamydospores were evident in 3 to 4 days, and in two weeks were in abundance. Oospores were formed by A3, A4 and A5 from the eleventh day onwards and were plentiful in 2 to 3 weeks.

Maize-meal agar.—The aerial growth was less than on the two former media in all the isolates and had a white woolly appearance. Sporangia and chlamydospores were numerous. Oospores were formed by the last three isolates only.

Carrot agar.—The aerial mycelium was much less than on other media but the production of sporangia and chlamydospores was on a par with that on the other media. Oospores were produced in greater abundance by the last three isolates only.

Potato dextrose agar.—Fluffy white aerial growth of moderate intensity was evident in all the isolates. Asexual reproduction was fairly abundant. Oospores began to develop one or two days later than on other media and were not so numerous.

There were differences in the rate of growth of the different isolates on the different media. On a comparative estimate the maximum intensity of production of sporangia and chlamydospores was on oats agar in all the isolates, though on frenchbean agar they were formed earliest. Oospores were produced only by the isolates A3, A4 and A5 and on carrot agar the largest numbers were produced, oats agar being the second best.

Reproduction.—The sporangia were the earliest to be formed in all the isolates. Differences were observed to some extent between those produced by the different isolates. The medium also appears to exert an influence on the size of these, as those formed on some media exhibited differences from those formed on other media in the same isolate. No consistent difference could be made out between the isolates either in the mode of formation of sporangia or in their shape. Chlamydospores were formed in all and these quickly followed the formation of sporangia. Here again differences were noticed in the size between those formed by some isolates and on certain media. Those formed by the isolates A1 and A2 were invariably bigger than those formed by the other three. Oogonia and oospores were produced only by the isolates A3, A4 and A5. The antheridia were always amphigynous. The differences in size of the oogonia and oospores formed by the different isolates and on different media were not significant. The mean size of these are given in Table I.

Contrary to the experience of most of the previous workers, oogonia and oospores were produced by single sporangial cultures by three of the isolates from areca. In all probability the reports of Coleman and Rosenbaum of the presence of oospores in pure cultures of the isolates from areca may be due to the presence of fertile homothallic strains. The results of the present studies show that more than one strain of the pathogen affects areca, some of them being homothallic and others heterothallic. The isolates A1 and A2 did not form oospores in single sporangial cultures but when paired with an isolate from betelvine these were readily produced indicating the heterothallic nature of the isolates. The measurements of the oogonia and oospores of the three isolates are in agreement with those recorded by Thomas and his co-workers (1947) for the oospores formed in paired cultures of different strains of *P. palmivora.*

Pathogenicity.—The host range of the five isolates was determined under green-house conditions at Coimbatore. The results are recorded in Table II.

All the isolates infected fruits of areca, *J. curcas* and *S. melongena,* leaves of coconut and seedlings of papaya. But in their ability to infect other

TABLE I

Measurements of Sporangia, Chlamydospores, Oogonia and Oospores in μ

Medium	Isolates																	
	A1		A2		A3				A4				A5					
	Sp.	Ch.	Sp.	Ch.	Sp.	Ch.	Oog.	Oosp.	Sp.	Ch.	Oog.	Oosp.	Sp.	Ch.	Oog.	Oosp.
Oats agar ..	46·5×31	46·5	50×37	46·5	37×21	32	25	22	40×35	32·5	31·5	23	46·5×34	31	25	22
French bean agar	43×31	48·0	46×34	48	45×31	40	28	22	43×31	37	28	23	45×31	37	23	20
Maize-meal agar	42×31	46·5	43×31	49	40×31	40	28	22	42×31	32·5	28	23	40×31	37	23	20
Carrot agar ..	46·5×37	48	46·5×37	46·5	43×34	37	25	22	46·5×37	40	25	23	45×37	40	25	22
Potato dextrose agar	37×25	34	48×37	50	46·5×36	40	25	22	48×39	40	28	22	46·5×37	40	23	20

Note.—Sp. = Sporangia ; Ch. = Chlamydospores ; Oog. = Oogonia ; Oosp. = Oospores.

TABLE II

Results of Inoculations on Different Hosts

Host	Part inoculated	Number inoculated	Number infected by				
			1	2	3	4	5
Areca catechu	Fruit	20	20	20	16	20	18
Agave wightii	Leaf	12	8	6	..	4	4
Bougainvillea spectabilis ..	,,	12	6	6	..	..	..
,, ...	Flower	12	6	6	..	..	..
Bryophyllum calycinum ..	Leaf	6	..	4	..	..	..
Capsicum grossum	Seedling	6	..	..	..	..	3
,, ...	Fruits	6	..	..	4	5	4
Carica papaya	Seedling	6	4	3	5	4	4
Cocos nucifera	Leaf bud	6	6	6	6	6	6
Jatropha curcas	Leaf	6	6	6	..	..	..
,, ...	Fruits	6	6	6	6	6	6
Lycopersicum esculentum ..	,,	12	..	6	..	..	..
Manihot glaziovii	Seedling	6	6	4	..	..	..
Ricinus communis	,,	6	..	..	..	..	..
Solanum melongena	Fruit	6	4	4	6	6	6
Solanum tuberosum	Tuber	6	..	..	..	..	..

hosts, differences were observed between the different isolates. A2 was able to infect more hosts than the others. The leaves and flowers of *Bougainvillea* and seedlings of *Manihot glaziovii* were affected by A1 and A2 alone. The leaves of *Bryophyllum* and fruits of tomato were infected by A2 alone. Only A5 affected chillie seedlings while A3, A4 and A5 were pathogenic on the fruits. The leaves of *A. wightii* were infected by all except A3.

DISCUSSION

Very interesting data have been obtained in these studies. Considerable differences were noticed among the strains of the pathogen causing fruit-rot of areca. Uppal and Desai (1939) were the first to demonstrate the existence of two physiologically different but complementary strains of the fungus on areca. These produced oospores in paired cultures. Our studies have shown that more than two strains of the pathogen are prevalent. It has

been definitely proved for the first time that fertile homothallic strains also occur on areca. The diseased fruits from which these isolates were obtained exhibited the presence of oospores in the pericarp, thus indicating that in nature also the sexual reproductive bodies are formed. These were of the same size and appearance as those formed on agar media. The prevalence of such homothallic strains is bound to give rise to more races and forms exhibiting differences in the virulence of the pathogen, host range, size of reproductive bodies and other characters. The fluctuations in the intensity of the incidence of the disease observed in different tracts during different seasons may not be entirely due to variations in the environmental factors but also be attributable to the prevalence of different strains of the pathogen differing in their virulence.

Mathews (1931) found that the best medium for obtaining the oogonia of *Pythium debaryanum* and *P. catenulatum* was to place cultures on thin slices of boiled carrot in a dark cabinet. In these studies it was found that carrot agar was a good medium for the formation of oospores, in fertile strains of the pathogen.

Summary

Five isolates of *P. palmivora* isolated from diseased areca fruits were under study. These exhibited differences in various characters. Sexual reproductive bodies were formed by some of the strains on the fruits in nature. These strains developed oospores in single sporangial cultures on agar media also. Carrot agar was found to be better suited for the formation of numerous oospores.

Literature Cited

Mathews, V. D. .. Studies on the genus *Pythium*. Univ. N. Carolina Press, 1931.

Narasimhan, M. J. .. *Phytopath.*, 1930, **20**, 201–14.

Rosenbaum, J. .. *J. Agric. Res.*, 1917, **8**, 233–76.

Thomas, K. M., Ramakrishnan, T. S., Soumini, C. K. and Balakrishnan, M. S. *Proc. Indian Acad. Sci.*, 1947, **25 B**, 147–63.

Uppal, B. N. and Desai, M. K. *Curr. Sci.*, 1939, **8**, 122–24.

Venkatarayan, S. V. .. *Phytopath.*, 1932, **22**, 217–28.

THE VACUOLE IN YEAST

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Introduction

Opinion is divided as to whether the vacuole is a permanent cell inclusion in animal as well as plant cells. The differences relate to the presence of a vacuole in living unstained meristematic and embryonic cells. Attempts have been made to homologise the vacuolar system of plant cells with the Golgi apparatus of animal cells (Zirkle, 1937) and the vacuole of yeast with the nucleus of plant cells (Wager and Peniston, 1910; Lindegren, 1949).

Historical Resumé

(*a*) *Vacuole in higher plants.*—The term "vacuole" was coined by Dujardin in 1841 to describe the small pulsating 'contractile' vacuoles of Protozoa (Zirkle, 1937, p. 1). Guilliermond (1941) mentions that the term "vacuole" is generally ascribed to watery inclusions in the cytoplasm of plant cells where numerous metabolic products tend to accumulate (p. 125).

It was generally believed before the time of deVries that the vacuoles in plant cells arise *de novo*. The origin of vacuoles was surmised to be the consequence of cell enlargement during tissue differentiation. Differing from the above view deVries suggested that even meristematic cells have plastid-like primordia, the *tonoplasts*, which enlarge by absorption of water into characteristic vacuoles during differentiation of tissues. The identical structures in meristematic cells are referred to by van Tieghem as 'hydroleucites'. Even at the time of deVries his view of the origin of vacuoles from 'massive tonoplasts' was not accepted by some who held the opinion that vacuoles originated only from pre-existing vacuoles. Though the term tonoplast was initially employed to designate plastid-like primordia its connotation has undergone a change and at the present day it refers only to the membrane surrounding a vacuole.

After reviewing the literature and on the basis of his own work Zirkle (1937) remarks that all meristematic cells contain vacuoles. The size and shape of vacuoles are said to be conditioned by the activity of the cytoplasm

(p. 5). From his extensive investigations, Guilliermond (1941) considers that the vacuoles in many plant cells may be permanent structures. Starting as small elements in meristematic cells they coalesce and enlarge after absorption of water during differentiation. It has been suggested that they have an intimate relation to tissue differentiation as well as secretory phenomena.

(*b*) *Vacuolar membrane.*—The phenomenon of isolation of vacuole gave rise to the suspicion that it is delimited from the cytoplasm by a semi-permeable layer. Chambers and Hofler (1931) showed by studies with a micromanipulator that the vacuolar membrane is "a highly cohesive and extensible fluid film of unappreciable thickness" (p. 352). Many of the investigators have experienced difficulty in demonstrating the existence of such a membrane with fixatives and stains. Guilliermond (1941) remarks that at least in mature cells there should exist a resistant semi-permeable membrane. The vacuole is said to have a luminous contour under the ultra-microscope (Guilliermond, 1941, Fig. 109, p. 164).

(*c*) *Vacuole in yeast.*—Wager and Peniston (1910) record an apparent disappearance of the vacuole of yeast under specific physiological conditions. They interpret these changes as the result of plasmolytic phenomena. Guilliermond (1941) considers that vacuoles may originate *de novo* in yeast. He records that not all vacuoles have a luminous contour under dark ground illumination but that the vacuolar boundary in yeast could often be surmised by the luminous lipid granules surrounding the vacuole (p. 165). Nagel (1946) describes the presence of a vacuolar membrane in *Saccharomyces* (p. 270).

Material and Methods

The material employed in the investigation was our control strain of yeast, BY 1. The nutrient medium contained 2% galactose (Merck.), 1% Difco Bacto-peptone and 0·5% Difco yeast extract. After the pH had been adjusted to 4·6–4·8, five ml. quantities distributed to sterile bacteriological test-tubes were sterilised at 15 lbs. for 15 minutes.

A few cells were inoculated from the stock agar culture into 5 ml. of barley malt wort of S.G. 1·020 and pH 4·6–4·8. When growth had proceeded for 16 hours at room temperature, which varied between 22–25° C., the material was shaken well and two loopfuls were transferred to the GPY medium. The cultures were examined every day for a period of 12 days.

A small quantity of the material from the GPY medium was put on a clean slide, mixed well to ensure the uniform distribution of the cells and mounted under a coverslip. The extra medium around the edges of the

coverslip was removed with filter-paper strips and the preparation was sealed with paraffin to avoid the movement of the cells. To get a correct idea of the various organelles, the living cells were studied under ordinary, phase contrast as well as dark ground illumination.

Under ordinary illumination only structures showing differences in light absorption could be distinguished. Fixation is usually resorted to to reveal organelles which could not be observed easily under ordinary illumination (Gatenby and Cowdry, 1928, p. 18). With a phase contrast microscope regions having slightly differing optical densities could be clearly distinguished. Under dark ground illumination only formed structures capable of reflecting light could be seen (Wolman, 1955, p. 80). For example, when a vacuole appears as if it has a membrane delimiting it from the cytoplasm, the question of its existence could easily be confirmed by the use of dark ground illumination.

Photographs were taken with a Leica attachment on 35 mm. Kodak 'Microfile' film. The negatives were enlarged to varying degrees.

OBSERVATIONS

Twelve-day culture.—The population is uniform in that all the cells are vacuolated (Photo 1). Though the majority have only a single vacuole, a small percentage of cells possess more than one (see Table). The size of the vacuole varies from cell to cell. It is small in some while in others it may occupy a major portion of the cell volume (Photos 1 and 2). The vacuolar boundary is distinct and distributed along its outer periphery occur few or many granules (Photos 1 and 2). In the majority of the cells, however, these granules are few in number the cytoplasm being almost free of visible inclusions.

When the identical cells are examined under phase contrast, the structures stand out with great clarity (Photo 3). The vacuole appears as if it has a well-developed membrane. Just outside the membrane could be seen the grains which vary in number or size from cell to cell. When the vacuole is focussed, the grains often are out of focus. As will be seen from Photo 3 the contour of the vacuole is not perfect. In some it appears lobed.

The general impression that one obtains by examination, especially under the phase contrast microscope, is that the vacuole may get constricted into a number of smaller vacuoles. Photo 4 shows two stages in the process. In cell A a constriction is visible and in cell B it is more pronounced. In the final stages of this process a canal-like connection may be seen between the vacuoles (Photo 5, A, B and C). There is very little glycogen in the cells

and the process of constriction of the vacuole seen in Photos 4 and 5 does not appear to be the consequence of the formation of glycogen. In the final stage where the cell is multi-vacuolate, it appears as if the vacuoles are separate (Photo 6, cell A) with no interconnections.

Under dark ground illumination the majority of the vacuoles appear to have a luminous contour. In Photo 7 the cell A has only one vacuole. In cells B and C two vacuoles differing in size from each other are present. Photo 8 illustrates the multi-vacuolate condition. The mother cell has two vacuoles while the bud has only one. Each of the vacuoles had a dancing body though in the photo only those in the adjacent vacuoles of the mother and bud are seen. In Photo 9 the cell has four vacuoles of different sizes and since each of them has a perfect luminous contour it seems probable that they are not interconnected. As may be seen the cells lying near the above have each only a single vacuole.

Effect of transfer to fresh media.—When the cells from the 12-day culture are stimulated for about 90–120 minutes by transfer to fresh media,—at a room temperature of 22–25° C.,—the granules and vacuoles disappear from view in the majority of the cells and the cytoplasm looks homogeneous. Photo 10 shows the stimulated cells under ordinary and Photo 11 under dark ground illumination. The cytoplasm appears free of any visible inclusions.

Five-day culture.—The picture seen is almost similar to that of the 12-day culture. Under the light microscope the cells are seen to possess well-developed vacuoles differing in size from cell to cell (Photo 12). Granules varying in number and irregular in size are seen scattered in the cytoplasm. In comparison with the 12-day culture, the major difference is the occurrence of very few multi-vacuolate cells (see Table). Though the vacuole appears as if it has a well formed membrane, confirmation of its presence is difficult with the phase contrast microscope (Photo 13).

Unlike in the 12-day culture only in a few of the cells does the vacuolar boundary appear luminous when examined under dark ground illumination (Photo 14). In others one can only surmise the position of the vacuole and its limits by following the movements of the luminous dancing body.

Three-day culture.—With the decreasing age of the culture increasing numbers of cells without vacuoles or grains could be observed (Photo 15, Table). There appears to be even a gradation in the size of the vacuole in different cells (Photo 15). Eleven to thirteen per cent. of cells are multi-vacuolate (Table). Relatively, the granules lying scattered in the cytoplasm

are fewer in number (Photo 15). Under phase contrast microscope no new structures could be located (Photo 16). The vacuolar boundary appears luminous only in a few cells (Photo 17 cell *A*). In some the vacuole appears as a dark area in a slightly luminous cytoplasm (Photo 17, cell *B*).

Twenty-four hour culture—(i) *Cultured at* 22–25° *C.*—When the temperature is between 22–25° C. very few cells in a 24-hr. culture show vacuoles or grains under ordinary (Photo 18) as well as the phase microscope. Under the phase microscope different regions of the cytoplasm show varying grades of density (Photo 19). Confirmation regarding the absence of grains in the cytoplasm is offered by Photo 20. As will be seen the cytoplasm is bereft of any light reflecting particles.

(ii) *Cultured at* 32° *C.*—During the month of March when the room temperature goes up to 32° C. a relatively larger percentage of cells show vacuoles or grains (Table). Photo 21 illustrates the appearance of cells grown at 32° C. under ordinary illumination.

Effect of transfer of cells from 24*-hr. culture to fresh media*—(i) *Cultured at* 22–25° *C.*—On stimulation of the 24-hr. crop of cells by transfer to fresh media, even those cells which showed grains or vacuoles appear free of any inclusions after the lapse of 40–90 minutes. The cytoplasm appears almost uniform. This condition is illustrated by the photos taken under ordinary (Photo 22), phase contrast (Photo 23) and dark ground illumination (Photo 24).

(ii) *Cultured at* 32° *C.*—Just as the presence of granules or vacuoles in a small percentage of cells in the 24-hr. crop is governed by the temperature at which the cells are grown, the changes observed on stimulation seem to be also governed by temperature. On stimulation for 40–90 minutes at 32° C. the small grains continue to persist in the cytoplasm of many of the cells even though the vacuoles disappear from view (Table). That regions of cytoplasm differ in optical density is illustrated by Photo 25 taken under phase contrast. A comparison of these with cells having typical vacuoles (Photos 1–9 and 12–17) would show that cytoplasmic areas of lesser density cannot be considered as vacuolar in nature.

Effect of fixation in Gram's iodine solution of stimulated cells from 24*-hr. culture at* 22–25° *C.*—The darker areas seen in the phase photographs of living cells appear to be regions occupied by glycogen. The picture obtained when the cells are stained in Gram's iodine solution (Photo 26) is superposable on the phase photomicrographs of living cells. Often glycogen occurs as a central mass surrounded by clear cytoplasm. The lighter areas in the phase photographs could be recognised after fixation with Gram's

Age of the culture	Temp. of Incubation	Cells without grains and vacuoles %	Cells with grains but without vacuoles %	Cells without grains but with vacuoles %	Cells with grains and vacuoles* %	Total No. of cells counted
1. 12 days ..	70° F. (± 5° F.)	0	1·6	0	98·4 (4·4)	500
	32° C.	0	1·2	0	98·8 (5·0)	,,
2. 5 days ..	70° F.	0	3·4	0·6	96·0 (3·4)	,,
	32° C.	0	2·4	0·8	96·2 (3·6)	,,
3. 3 days ..	70° F.	3·0	9·2	1·6	86·2 (13·2)	,,
	32° C.	1·6	10·6	2·2	85·6 (11·6)	,,
4. 24-hr. Unstimulated	70° F.	71·8	19·6	2·6	6·0	,,
	32° C.	60·6	23·2	3·2	(2·0) 13·6 (4·4)	,, ,,
5. 24-hr. .. Stimulated	70° F.	88·4	9·0	0·6	2·0 (0·0)	,,
	32° C.	73·2	21·2	1·6	4·0 (1·6)	,,

The percentages given within brackets in the column marked with an asterisk refer to cells with more than one vacuole.

iodine as regions of cytoplasm where glycogen is absent. They should not therefore be mistaken for typical vacuoles illustrated in 3, 5 and 12-day cultures.

Effect of washing and hydrolysis of Gram fixed cells.—When cells fixed in Gram's iodine for one hour are washed under tap for two hours and examined under ordinary illumination the regions originally stained by iodine and indicating the presence of glycogen appear lighter than the rest of the cytoplasm. A distinct spherical to oval structure is also revealed as a result of the above procedure (Photo 27). Examination of the washed

cells with the phase contrast is rendered difficult owing to the incomplete removal of iodine. If the cells fixed in Gram's iodine are hydrolysed for 8 minutes in N HCl at 60° C. after a long wash in tap water and two washes in distilled water and then treated with a dilute solution of sodium thiosulphate to remove the traces of iodine, the body seen after washing following fixation (Photo 27) stands out clearly and the region originally occupied by glycogen appears as a light area under the phase microscope (Photo 28). The body revealed on fixation is reminiscent of the nucleus of yeast described by most authors. Contrary to the observations of Wager and Peniston (1910, p. 50) when a vacuole is not present in a living cell, Gram's iodine does not reveal a vacuole.

DISCUSSION

(i) *The question of permanency of the vacuole.*—With the increasing age of the culture almost all cells appear vacuolated. But the transfer of yeast crop from an old culture to fresh medium results in the apparent disappearance of the vacuole in the majority of the cells after the lapse of about an hour. This is especially marked when the cells from a 24-hr. culture stimulated with fresh medium are examined 40–90 minutes after transfer. The majority of the cells do not show either vacuoles or grains (Photos 22 and 23). The progressive decrease in the number of vacuolated cells with the decreasing age of the culture taken in conjunction with the observation of the apparent disappearance of the vacuoles and grains after a period ranging between 40–120 minutes after transfer to fresh medium, makes one doubt whether a vacuole could after all be considered as a permanent organelle of the yeast cell.

It was indicated earlier that opinion is divided on the presence of vacuoles in meristematic cells. Even in those cases where demonstration of vacuolar primordia have been claimed, such primordia have little resemblance to the vacuoles in cells of differentiated tissues. It would be well to remember Guilliermond's (1941, p. 222) conclusion that a colloidal granule secreted by the cytoplasm may later enlarge into a vacuole by absorption of water. Probably as a corollary it is that he remarks that vacuoles may often arise *de novo* in yeast.

Guilliermond illustrates (1941, Fig. 126, p. 180) the origin of the bud vacuole in two different ways. The vacuole of the mother cell may put out a projection into the bud which by fragmentation at its tip gives rise to the vacuole of the bud. On the other hand he records that the vacuole of the bud may originate *de novo* even when the mother cell has a well formed

vacuole. The observations recorded in this paper support Guilliermond's contention that the vacuole may often originate *de novo* in yeast.

(ii) *The vacuolar membrane.*—Though the vacuoles present in a 3-day culture could be seen under ordinary as well as phase contrast microscopes, examination under dark ground illumination reveals that only in a few could the vacuole be definitely identified by its luminous contour. Often the limits of the vacuole have to be surmised either by the range of movement of the dancing body or the slightly luminous nature of the cytoplasm. Under dark ground illumination only formed structures capable of reflecting light could be observed. The luminous contour when present could be taken as evidence for the presence of a membrane surrounding the vacuole. As Photos 7, 9, 14 and 17 would indicate, more and more of vacuolated cells exhibit luminous contours to their vacuoles with the increasing age of the culture. It is to be presumed, therefore, that as a result of aging, the vacuoles become slowly delimited from the cytoplasm by a well formed membrane.

(iii) *The number of vacuoles in a cell.*—The questionwhether a multi-vacuolate condition precedes the univacuolate stage was kept in view. As could be seen from the photographs taken under different types of illumination, the majority of cells possess only a single vacuole. Cells with more than one vacuole are relatively few in number. Therefore it seems likely that the multi-vacuolate condition is the result of fragmentation of the single vacuole.

It is known from continuous observations on living yeast cells that the vacuole exhibits changes in its configuration from time to time. Their regular spherical contour may become irregular or angular and the vacuole itself may increase or decrease in size (Guilliermond, 1941, p. 177; Lindegren, 1949, p. 5–2). That similar changes are happening in the yeast which forms the subject of this study could be made out from the photomicrographs 3 and 5. The absence of any connection between the vacuoles in the same cell could be surmised from observations under dark ground illumination when each vacuole has a well formed membrane (Photos 7, 8 and 9).

(iv) *Significance of the absence of a vacuole in some cells.*—Examination of stimulated cultures 40–90 minutes after transfer to fresh medium shows that the majority of the cells have absolutely clear cytoplasm without any vacuoles or grains. Such a phase in the growth-cycle of yeast is reported by Wager and Peniston (1910). They transferred yeast to fresh Pasteur's solution and found at the end of an hour that the vacuole " has apparently disappeared from most of the cells " (Wager and Peniston, 1910, Fig. 13,

pp. 49–50). Treatment with iodine solution is said to reveal its presence and on that basis they interpret the disappearance of the vacuole as a plasmolytic phenomenon.

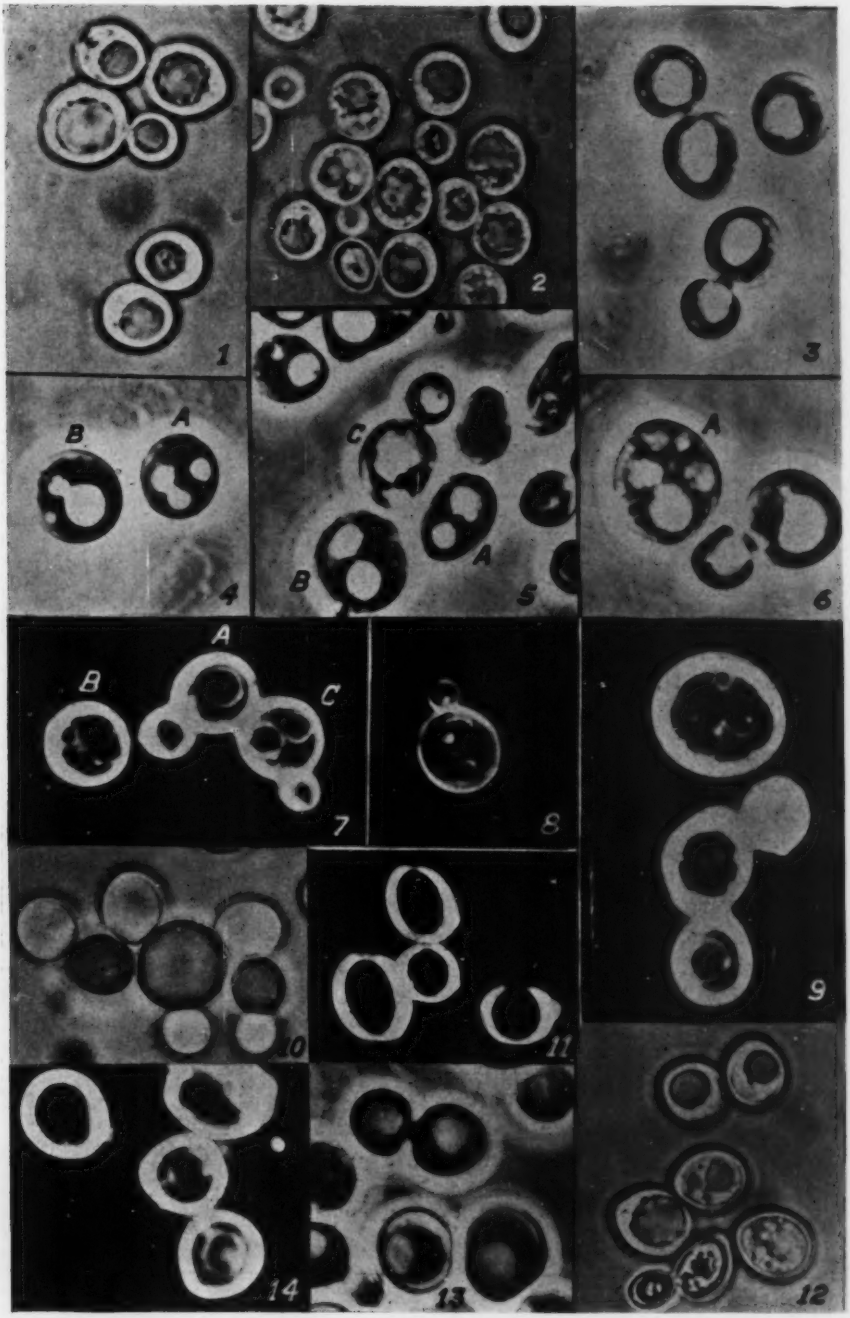
Guilliermond (1941, p. 180) cites the observations of Cassaigne that often the vacuole of the bud may arise *de novo*. This view conflicts with that of Wager and Peniston (1910) and since in the stimulated cells of BY 1 no vacuole could be seen under phase contrast (Photo 23), it has to be presumed that during a certain period after transfer of cells to fresh media, they do not have any vacuoles. Using a quartz microscope and ultra-violet illumination, Caspersson and Brandt (Lee and Hanson, 1947) observed the disappearance of the granules on stimulation of the cells. On dissolution of the grains the nucleic acids present in the grains get uniformly distributed in the cytoplasm.

In the meristematic cells of higher plants vacuoles of the type seen in differentiated cells are lacking. If comparison with the condition in higher plants is permissible, then, in yeast also, there is a stage when they could be compared to the meristematic cells (Subramaniam, 1952). But as has been shown, such a phase is a transitory one. A perusal of the photos of samples from cultures ranging in age from 24-hr. to 12-days would suggest that with the progress of time the cells develop (i) grains and (ii) vacuoles. During the process of aging, the vacuole develops (iii) a membrane and inside it (iv) sometimes a dancing body puts in its appearance.

Can these changes be considered as an example of *differentiation-in-time*? Darlington (1932) remarks that what appears as *differentiation-in-space* in higher organisms becomes one of *differentiation-in-time* in Protista. That does not however imply that there is no differentiation-in-time in metazoa and metaphyta (see also Conklin, 1925). He cites protandrous mollusca as an example of differentiation-in-time (p. 13), where an individual producing only male gametes later produces purely female ones. If the concept that differentiation is common to both multicellular and unicellular organisms is considered valid, then, while young yeast cells lacking vacuoles as well as grains could be compared to meristematic cells, those from older cultures which have vacuoles as well as grains have to be considered as analogous to cells of differentiated tissues. Such a distinction, it would be realised, has an important bearing in any analysis of the cytology of yeast based on fixed and stained preparations.

SUMMARY

1. The vacuole was studied under ordinary, phase contrast and dark ground illumination in yeast of varying ages. With the increasing age of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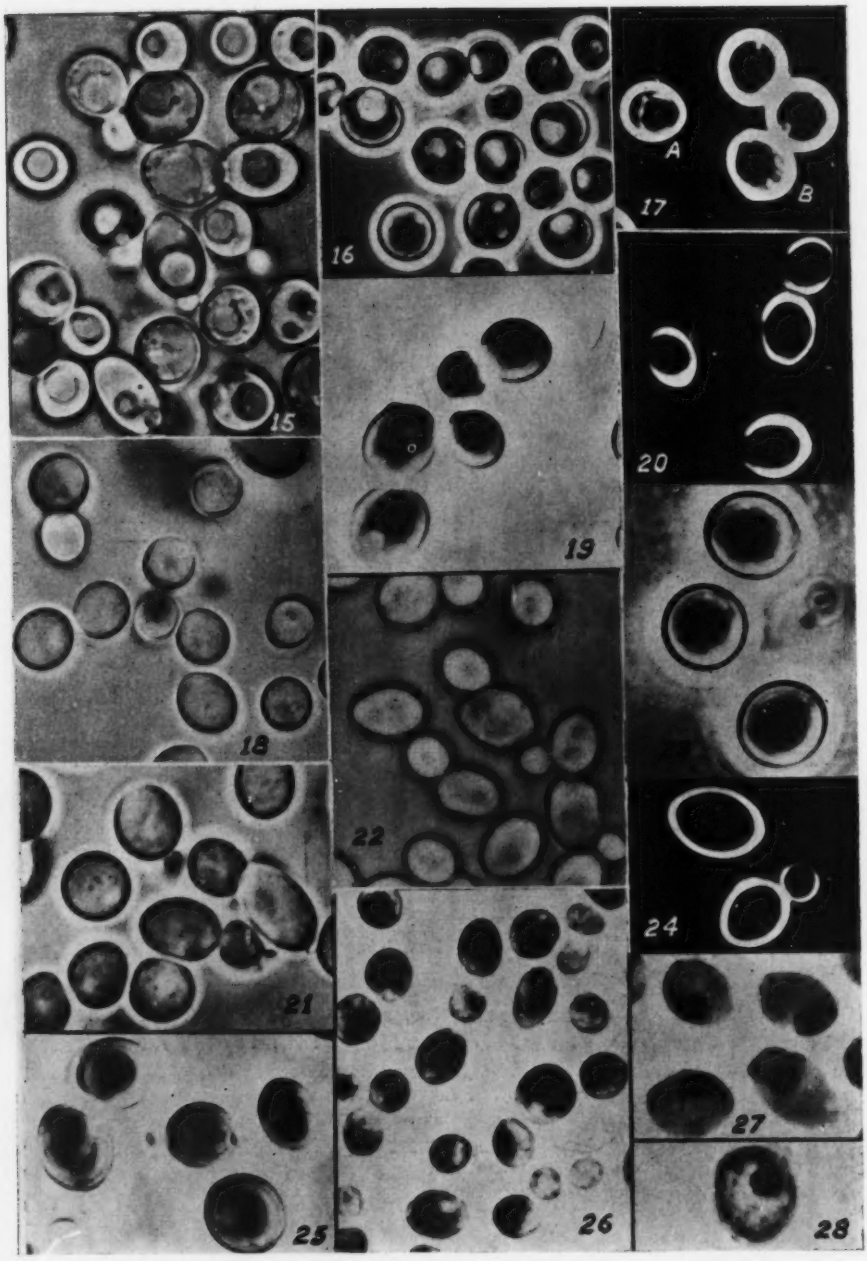
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the culture almost all cells appear vacuolated and more and more cells exhibit luminous contours to their vacuoles.

2. On transfer to fresh media the vacuoles and grains disappear from view and the cytoplasm looks homogeneous. During such a phase in its growth-cycle, yeast appears comparable to meristematic cells.

3. During the process of aging, the cells exhibit granules and vacuoles and the vacuole comes to possess a membrane delimiting it from the cytoplasm and sometimes a dancing body inside. Can these changes be considered as an example of differentiation-in-time in yeast?

ACKNOWLEDGMENT

I am very grateful to Dr. M. K. Subramaniam for his criticisms and encouragement.

REFERENCES

1. Carleton, H. M. and Leach, E. H. — *Histological Technique*, Oxford Univ. Press, 1938.
2. Chambers, R. and Hofler, K. — "Micrurgical Studies on the Tonoplast of *Allium cepa*," *Protoplasma*, 1931, **12**, 338–55.
3. Conklin, E. G. .. "Cellular Differentiation," in *General Cytology*, Ed. E. V. Cowdry, 1925, 537–607.
4. Darlington, C. D. .. *Recent Advances in Cytology*, 1932, J. and A. Churchill, London.
5. Gatenby, J. B. and Cowdry, E. V. — *Bolles Lee's Microtomist's Vade Mecum*, 1929, J. and A. Churchill, London.
6. Guilliermond, A. .. *The Cytoplasm of the Plant Cell*, 1941, Chronica Botanica Co.
7. Lee, R. and Hanson, W. A. — *Protomorphology*, 1947, Lee Foundation for Nutritional Research, Milwaukee, Wis.
8. Lindegren, C. C. .. *The Yeast, its Genetics and Cytology*, 1949, Educational Publishers, St. Louis.
9. Nagel, L. .. "A Cytological Study of Yeast," *Ann. Mo. Bot. Gdn.*, 1946, **33**, 249–88.
10. Subramaniam, M. K. .. "On the Identification of the Various Structures in the Yeast Cell," *J. Ind. Inst. Sci.*, 1952, **34**, 11–23.
11. Wager, H. .. "The Nucleus of the Yeast Plant," *Ann. Bot.*, 1898, **12**, 499–543.
12. ——— and Peniston, A. — "Cytological Observations on the Yeast Plant," *Ibid.*, 1910, **24**, 45–83.
13. Wolman, M. .. "Problems of Fixation in Cytology, Histology and Histochemistry," *Internat. Rev. Cytology*, 1955, **4**, 79–102.
14. Zirkle, C. .. "The Plant Vacuole," *Bot. Rev.*, 1937, **3**, 1–30.

DESCRIPTION OF PHOTOMICROGRAPHS

(i) *Cells of BY* 1 *from twelve-day cultures* (*Unstimulated*)

PHOTO 1. Cytoplasm with a few grains and a vacuole (Ordinary illumination).

PHOTO 2. Vacuole and many grains (Ordinary illumination).

PHOTO 3. A single large vacuole. Vacuolar boundary distinct and lobed (Phase Contrast).

PHOTO 4. Vacuoles showing constrictions (Phase Contrast).

PHOTO 5. Vacuoles in the final stages of constriction showing the canal-like connection between the daughter vacuoles (Phase Contrast).

PHOTO 6. Cell A shows the multi-vacuolate condition (Phase Contrast).

PHOTO 7. Cell A has a single vacuole. Note the differences in the size of the vacuoles in each of the cells, B and C (Dark ground).

PHOTO 8. Multi-vacuolate condition of some cells. The mother cell has two vacuoles while the bud has only one. The dancing body is seen in two of the vacuoles (Dark ground).

PHOTO 9. Cell having four vacuoles differing in size from one another.

(ii) *Twelve-day culture stimulated for* 90–120 *minutes with fresh medium*

PHOTO 10. Cells without vacuoles or grains (Ordinary illumination).

PHOTO 11. Cytoplasm free of any inclusions (Dark ground).

(iii) *Five-day culture* (*Unstimulated*)

PHOTO 12. Vacuolated cells with grains (Ordinary illumination)

PHOTO 13. Vacuolated cells (Phase Contrast).

PHOTO 14. Only some vacuoles have luminous contours (Dark ground).

(iv) *Three-day culture* (*Unstimulated*)

PHOTO 15. Vacuoles and grains in the cytoplasm (Ordinary illumination).

PHOTO 16. Vacuolated and non-vacuolated cells (Phase Contrast).

PHOTO 17. Only some vacuoles have luminous contours (Dark ground).

(v) *Twenty-four-hour culture* (*Unstimulated*, 22–25° *C.*).

PHOTO 18. Cells with few granules (Ordinary illumination).

PHOTO 19. Regions of cytoplasm differ in optical density (Phase Contrast).

PHOTO 20. Cells without vacuoles or grains (Dark ground).

(vi) *Twenty-four hour culture* (*Unstimulated*, 32° *C.*)

PHOTO 21. Cytoplasm showing regions of differing density as well as grains (Ordinary illumination).

(vii) *Twenty-four-hour culture* (*Stimulated*, 22–25° *C.*)

PHOTO 22. Cytoplasm bereft of any inclusions (Ordinary illumination).

PHOTO 23. Cells without grains or vacuoles (Phase Contrast).

PHOTO 24. Cells free of formed structures (Dark ground).

(viii) *Twenty-four-hour culture* (*Stimulated*, 32° C.)

PHOTO 25. Regions of cytoplasm differ in optical density (Phase Contrast).

(ix) *Stimulated cells from Twenty-four-hour culture fixed in Gram's iodine* (22–25° *C.*)

PHOTO 26. Areas of the cytoplasm containing glycogen (Ordinary illumination)

PHOTO 27. Spaces left in the cytoplasm on washing out the fixative (Ordinary illumination).

PHOTO 28. Gram fixed cells washed, hydrolysed and treated with sodium thiosulphate to remove traces of iodine. The areas originally occupied by glycogen are lighter. The nucleus is seen (Phase Contrast).—

Range in cell size 2·9–8·7 $\mu \times 3 \cdot 6$–11·9 μ.

LEAF BLIGHT DISEASE OF *CITRUS ACIDA* VAR. *VARIEGATA*

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(Communicated by Dr. S. N. Das-Gupta, F.A.SC.)

Introduction

During the rainy season of 1946, Dr. S. N. Das-Gupta brought to the notice of the author a tree of *Citrus acida* var. *variegata* in a local garden that showed a large number of blighted leaves. The disease differed from the wither-tip of citrus plants described from Northern India (Choudhury and Singh, 1930), both in the symptoms and the effect of the disease, hence a detailed study of the problem was taken up.

Citrus acida var. *variegata* is a comparatively rare variety of this species and as the name indicates, the leaves of this plant show a brilliant yellow green variegation. This character makes this variety an important ornamental plant.

Similar disease was observed in trees of the same variety in other local gardens and subsequently in the departmental garden when the plants were grown for experimental studies. Materials from all these sources were collected for isolations.

Symptoms

The disease is noticeable at all times of the year but is most conspicuous towards the end of the rainy season and the beginning of the winter. By mid-winter the infected leaves gradually fall away and the trees assume a healthier appearance. Freshly infected leaves are again seen on the advent of spring in the month of February.

The fungus attacks leaves of all sizes from young to fully mature ones, but it is the younger leaves, which are completely or partially devoid of chlorophyll, that are most susceptible to the attack.

The first external symptom of the disease is the slight discolouration followed by browning of the margin or the tip of the leaf (Pl. XIV, Fig. 1).

With advance of the infection the brown area increases. Occasionally a number of concentric rings are evident in the brown area formed by the developing acervuli (Pl. XIV, Fig. 6), which are pinkish orange or flesh coloured in the beginning and turn dark later on. In all the cases observed so far the infection starts from the chlorotic zone though it may extend to the green parts also. In advanced stages of the disease the dead tissue usually falls away. Young leaves may sometimes retain the dead areas along the margins and exhibit a distorted appearance of the remaining portion of the lamina.

The fungus has been found to attack only the leaves and so far no infection of the stem or fruits has been observed.

Isolation of the Causal Organism

Leaves showing different stages of the disease, right from those showing the earliest symptom to fully infected leaves showing numerous acervuli, were utilised for isolations.

The leaves were surface sterilised by swabbing them with a piece of cotton dipped in ·1% mercuric chloride solution and then were washed in three to four changes of sterile distilled water. Small pieces of the infected portion of the leaves showing early stages of infection were cut by a sterilised scalpel and were inoculated in Brown's synthetic tube slants. In others, conidia from the acervuli were inoculated by the needle.

In every case the same fungus was isolated which was later identified to be *Colletotrichum glœosporioides* Penz.

Inoculation experiments were performed with this fungus both under laboratory conditions and in the field.

Inoculation Experiments

Material and Method

The following set of inoculation experiments were performed:—

(*a*) On detached leaves in sterile moist chambers.

(*b*) On leaves of potted plants under laboratory conditions.

(*c*) On leaves of plants in the field.

(*d*) Inoculation of younger twigs and stem, and other species and varieties of citrus.

Healthy detached leaves for the inoculations were obtained from plants growing in the Government Horticultural Gardens, Lucknow.

For inoculations under laboratory conditions, 20 plants were raised in pots from cuttings. An effort made to raise the plants from seed (about 100 in number) proved unsuccessful since all the seedlings were devoid of chlorophyll and hence could not survive long.

Under field conditions inoculation experiments were performed on 5 plants growing in the departmental garden that were raised from healthy cuttings obtained from the plants in Government Horticultural Gardens, Lucknow. The shoot inoculations of *Citrus acida* var. *variegata* plants as also the inoculations of other species and varieties of Citrus was done following similar methods both under laboratory and field conditions.

The leaves to be inoculated were first surface sterilised by swabbing with ·1% mercuric chloride and were then rinsed with sterile distilled water. Two methods of inoculation were used:

(i) Spraying the spore suspension of the pathogen on the leaves.

(ii) Local application of the spores on the leaf surface.

The inoculations were done both on the upper and lower surfaces of the leaf either with or without injury. Since the survey results as also the preliminary pathogenicity tests showed that infection always started from the chlorotic zones, leaves showing complete or partial absence of chlorophyll were utilised for the purpose and the inoculations were made in the chlorotic areas.

All the inoculation experiments were performed during rainy season.

(*a*) *Inoculation of detached leaves.*—A preliminary set of inoculation experiment was carried on detached leaves. The leaves were inoculated by the method described earlier and were kept in sterile moist chambers. Nearly all the leaves got infected irrespective of the mode of inoculation and acervuli appeared all over the surface in seven to eight days after inoculation at room temperature (26° C.–32° C.).

(*b*) *Inoculation of leaves of potted plants under laboratory conditions.*—Potted plants were used for this set of experiment. After the inoculations the plants were watered well and the pots were kept under bell jars, and after 4 days when water-soaked areas started appearing on the surface of

the inoculated leaves, the plants were kept out in the open and were watered regularly. The following table gives the results of these inoculations:—

TABLE I

Showing the result of inoculation of Citrus acida *var.* variegata *leaves under laboratory conditions with spores of* C. glœosporioides

No. of leaves inoculated	Surface	Mode of inoculation	With or without injury	No. of leaves getting diseased	% of leaves getting diseased
40	Upper	Spraying	Without	13	32·5
40	Lower	Spraying	Without	11	27·5
40	Upper	Local inoculation	With	26	65
40	Upper	,,	Without	21	52·5
40	Lower	,,	With	29	72·5
40	Lower	,,	Without	18	45

It will be clear from the above table that under laboratory conditions both the methods of inoculation, *i.e.*, of spraying the spore suspension and the local inoculation, were successful. With the same mode of inoculation there is no appreciable difference from the upper and lower surfaces of the leaves. Local inoculation proved to be a more effective method and injury tends to increase the rate of infection. While 45% and 52% infection was obtained from lower and upper surface without injury, the percentage rose to 72·5% and 65% respectively with injury.

While the foregoing inoculation experiments were performed by spores from culture, one set of experiments using 50 leaves was tried directly from spores of infected leaves. Following similar methods inoculations were performed both with and without injury and successful infection of 21 leaves was obtained showing thereby the infectivity of the conidia obtained directly from the diseased leaves.

(*c*) *Inoculation of leaves under field conditions.*—This set of experiments was carried out on leaves of plants in the field. These inoculations were performed during the evening which was followed by a cool and moist night. The leaves were further sprayed with sterile water for the following 3 days

to provide moisture for the growth of the pathogen. The results are given in Table II.

TABLE II

Showing the results of inoculation of Citrus acida *var.* variegata *leaves with spores of* C. glœosporioides *under field conditions*

No. of leaves inoculated	Surface of the leaves utilised	Mode of inoculation	With or without injury	No. of leaves getting diseased	% of leaves getting diseased
80	Upper	Spraying	Without	7	9
80	Lower	Spraying	Without	9	11
50	Upper	Local inoculation	With	13	20
50	Upper	,,	Without	11	22
50	Lower	,,	With	18	30
50	Lower	,,	Without	8	10

Table II will show that successful infection of the leaves could be obtained under the field conditions also though the percentage of infection was much less. Here also injury tends to favour infection and upto 30% infection is obtained with injury from the lower surface while only 10% is obtained without it.

A comparison of Tables I and II will show that with leaves *in vivo* the percentage of infection is as high as 72·5% under the laboratory conditions while the maximum obtained in the field condition is only 30%. Apart from other factors that may be responsible, moisture seems to be an important factor for this difference as the plants in the laboratory get more or less a saturated atmosphere inside the bell jar for 4 days after the inoculation.

Some difference was exhibited in the symptoms of the leaves that were inoculated with spraying and those where local inoculation was performed. In case of spraying, as there were various foci of infection, the whole leaf surface was covered with acervuli in eight to ten days, while in leaves where inoculum was placed on localised spots, the symptoms observed were more like the naturally diseased leaves. The first symptom which appeared on

the fourth day was the appearance of small water-soaked area round the inoculum. This gradually increased in size and turned brown killing the tissue of the infected part. The acervuli started appearing on the seventh or eighth day which were pinkish orange colour in the beginning and later turned dark.

(*d*) *Inoculation of younger shoots and other species and varieties of Citrus.*—As *Colletotrichum glœosporioides* has long been known to cause wither-tip of young shoots, in spite of the fact that no shoot infection was observed in nature, inoculation experiments on young healthy shoots of *Citrus acida* var. *variegata* were performed to find out the effect but the experiments gave negative results.

Earlier Choudhury and Singh (1930) had reported a strain of *Colletotrichum glœosporioides* which infected different species and varieties of Citrus. Fawcett (1936) has also reported similar result on different species and varieties of Citrus with a single strain of the pathogen. With these results in view, inoculation experiments were tried with this fungus on (i) a normal green healthy variety of *Citrus acida*, and (ii) a variety of *Citrus aurantium*.

Following the methods described earlier leaf inoculations were tried both in the field and under the laboratory conditions but no success was obtained in infecting the leaves of these varieties.

MORPHOLOGY OF THE FUNGUS

In Culture

(*a*) *The mycelium.*—In culture on Brown's synthetic medium in the beginning there is a dense growth of the white mycelium for the first 4 to 5 days after inoculation. Later the dense character of the mycelium gradually disappears and the mycelium becomes sparse. The acervuli make their appearance in the culture after about 6 to 7 days. Simultaneously copious formation of conidia from sporodochia is also visible at different spots in the medium. These spore masses are flesh coloured.

(*b*) *The acervuli.*—The size of acervuli in culture ranged from 200 to 500 μ with a mean of 445 μ.

The spore size varied from 10 to 28 μ by 4 to 7·5 μ with a mean of 16 μ by 5·5 μ. (The mean has been calculated on the measurement of 200 spores.)

Acervuli in natural condition

The acervuli occur on both upper and lower surfaces of the leaves but they are comparatively more abundant on the lower.

The colour of the acervuli is pinkish orange or flesh coloured in the beginning and may later turn dark. In highly infected portions of leaf it is not uncommon to find pink acervuli occurring interspersed with dark ones while towards the margin mostly pink ones are found. The shape and size of the acervuli varied greatly. Setæ were mostly absent and were found in very rare cases. The size of acervuli ranged from 75 μ to 280 μ in diameter with a mean of 148·5 μ.

The measurement of the spores varied between 10 to 22 μ by 4·5 to 6·5 μ with a mean of 14·6 μ by 5·1 μ.

The fungus has been identified as a new strain of *Colletotrichum glæosporioides* Penz.

Since the morphological and physiological characters of this causal organism were very much different from any description given of *Colletotrichum glæosporioides*, both the infected leaves and the material in culture were sent to Dr. Westerdijk who identified the pathogen as *Colletotrichum glæosporioides* although on the basis of the dry specimens the fungus could not be placed definitely either as *Colletotrichum glæosporioides* or as *Gloœosporium limetticolum*.

Discussion

Colletotrichum glæosporioides Penz., the cause of the wither-tip or anthraconse disease of Citrus, is known to affect various species and varieties of Citrus plants.

The strain of *Colletotrichum glæosporioides* causing the leaf spot disease described in this paper shows certain marked variation from other known and commonly described strains of this fungus both in its morphological and physiological characters.

According to Rolfs (1904) the acervuli and spore measurements of *Colletotrichum glæosporioides* are—acervuli 90–240 μ and spores 10–16 μ by 5–7 μ while in this strain of fungus the size of the acervuli varies from 75 to 280 μ in diameter on the leaf surface and those of the spores from 10 to 22 μ by 4·5 to 6·5 μ. The size of the acervuli in culture reaches as big as $\pm$ 500 μ. However, Burger (1921) has described the variability of this fungus and according to his description it falls within the limits of the species. He described *Colletotrichum glæosporioides* as a polymorphic species made up of a number of strains and classifies them in five groups. These strains differ characteristically from each other. The features of this isolate are within the range of *Colletotrichum glæosporioides* as given by Burger.

The pathogenic nature of the fungus has been successfully established both under the laboratory and field conditions though the percentage of infection under field conditions is lower than in laboratory. Apart from other conditions that may be responsible, moisture seems to affect the percentage of infection. Under similar conditions injury increased the rate of infection.

Both in natural condition and in experimental studies so far observed, it was the chlorotic leaves or chlorotic part of the variegated leaves that was susceptible to infection. Fresh infections seem to start always from the chlorotic zone though it may extent to the green parts later on.

Unlike the results obtained by Chaudhury and Singh (1930), this strain of the fungus showed great specialization in its attack. It infects only the plants of *Citrus acida* var. *variegata* and no other variety or species of Citrus has been so far found attacked by it either in nature or by artificial inoculation. In the infection of that particular variety too, it differs greatly from other commonly known strains of *Colletotrichum glœosporioides* in that it failed to infect any part of the plant other than the leaves.

SUMMARY

A leaf spot disease of *Citrus acida* var. *variegata* has been recorded. The disease is most conspicuous during rainy season and at the fall of winter in spring.

The symptoms of the disease have been described in detail.

Successful inoculation experiments have been performed both under field and laboratory conditions. Spores both from culture as well as those occurring in the acervuli of the leaves could bring about infection of the leaves. It was found that injury increased the rate of infection.

The morphological character of the pathogen has been described and the fungus has been identified as a new strain of *Colletotrichum glœosporioides* Penz. differing in several characters from other strains.

ACKNOWLEDGEMENTS

The author is grateful to Dr. Joh Westerdijk of Netherlands, for identification of the fungus, and to Professor S. N. Das-Gupta, PH.D., D.SC. (Lond.), D.I.C., for his interest in the problem.

LITERATURE CITED

*1. Burger, O. F. .. "Variation in *Colletotrichum glœosporioides*," *Jour. Agr. Res.*, 1921, **20**, 723–36.

2. Chaudhury, H. and Singh, G. "The wither-tip disease of Citrus plants—I," *Jour. and Proc. Asiatic Soc. Bengal*, 1930, **26**, 523–32.

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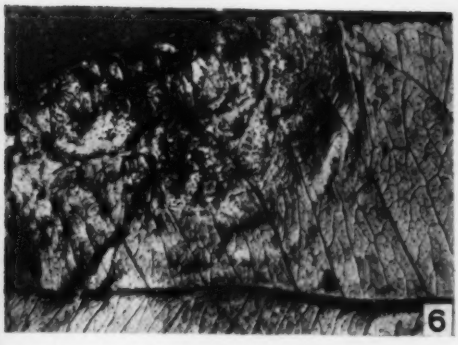
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3. Clausen, R. E. .. "A new fungus concerned in wither-tip of varieties of *Citrus medica*," *Phytopathology*, 1912, **2**, 217–34.

4. Fawcett, H. S. .. *Citrus Diseases and Their Control*, McGraw-Hill Publications, New York, 1936.

*5. Rolfs, P. H. . "Wither-tip and other diseases of Citrus trees and fruits," *U.S. Dept. Agr. Bur. Plant Indus. Bul.*, 1904, **52**, 20.

Explanation of Plate

Plate XIV

Fig. 1. Early stage of infection showing little browning of the tip and the margin.

Fig. 2. Advanced stage of the disease where large area of the leaf has been infected but the acervuli have not appeared so far. Zoning can be seen.

Figs. 3 & 4. Advanced stages of the disease when the infected portion of the leaf is entirely killed.

Fig. 5. A specimen showing all the leaves infected by artificial inoculation. × ½ natural size.

Fig. 6. A part of an infected leaf showing the zonal arrangement of the acervuli which are seen as black dots. × ca. 6.

Fig. 7. Photomicrograph of condia of *Colletotrichum glœosporioides* from culture, × 300.

* Literature not consulted in original. Extracts taken from the correspondence with Dr. Westerdijk.

AP ER CHROMATOGRAPHY IN FISH TAXONOMY

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Introduction

The correct identification of species is of primary importance to the fishery biologist in his task of assessing the composition of any fishery stock. Conventional taxonomic methods are undoubtedly of great value for this purpose but they have their limitations especially when the specimens under study are too small or when geographical and other factors, unidentified as yet, complicate the picture. Very often the discontinuity in morphological features may not be adequate or the recognition characteristics may be insufficient and in these cases information might well be supplemented by additional methods.

In recent years the technique of paper partition chromatography has been applied with success as an aid in the taxonomy of fishes (Buzzati-Traverso and Rechnitzer, 1953), land snails (Kirk *et al.*, 1954), mosquitoes (Micks, 1954) and fruit flies (Buzzati-Traverso, 1953). The method consists essentially in drying a bit of tissue on filter-paper, dissolving out constituents of the tissue with a chemical solvent travelling along the paper by capillary action, and, after removal of solvent, identifying the linearly separated constituents by the use of suitable indicators or fluorescent light. Buzzati-Traverso and Rechnitzer employed this method in their study of tissue of *Paralabrax clathratus*, *P. maculatofasciatus* and *Hysterocarpus traski*. They found ninhydrin-positive patterns of a certain tissue taken from various specimens of the same species to be constant, irrespective of the size or age of the fish, while patterns obtained from muscle of different species showed constant but easily recognizable differences. The significance of these results to marine fishery investigations was realised by the Californian Marine Fishery Research Committee (1952–53) who initiated a programme of work on the application of paper partition chromatography to the study of subgroups and migratory habits in sardine populations along the Californian coast. In the present article the possibilities are discussed, on the basis of experiments, of a similar approach to the study of fish populations in the Mandapam area.

Material and Methods

Preliminary Experiments

Fishes were obtained from commercial catches. The fish-muscle samples were spotted on filter paper-according to the instructions of Buzzati-Traverso and Rechnitzer. For chromatography, Whatman No. 1 filter-paper was used throughout.

In the first sets of experiments, the apparatus employed was the same as that indicated by Krishna Pillai (1953) for the preparation of ascending chromatograms. The solvent system was made up of *n*-butanol, acetic acid and water, mixed in the ratio of 40: 10: 50, v/v. The chromatograms were sprayed with a 0·2% solution of ninhydrin in rectified spirit. The patterns from *Sardinella albella* and *Sardinella gibbosa* were equivocal though differences could be noted in widely different species like *Hemirhamphus georgii* and *Atherina* sp. (Fig. 1).

Final Procedure

Details of subsequent experiments are given in Table I.

Table I

Paper Chromatography of Fish Muscle: Experimental Details

Chromatographic method	Size of filter-paper	Solvent	Paper presatd. for (hrs.)	Colour† reagent
Ascending ..	30×13 cm.	*n*-BuOH/AcOH/H_2O (100: 22: 50, v/v)	1·5	Ninhydrin
Disk* (Two runs) ..	12·5 cm. diam.	Propanol-ammonia (1%) (4: 1, v/v)	0·5	Ninhydrin and rectified spirit containing 5% pyridine

* *Cf.* Giri (1953). The solvent was contained in a Petri dish (6·3 cm. diam.) and the chromatograms were developed in between Petri dishes (15 cm. diam.).

† Dip method; patterns traced after keeping chromatograms for a few minutes between 60° and 70° C.

The sardines used ranged from 89 to 126 mm. in total length. Two samples from *S. albella* and two from *S. gibbosa* were spotted on each sheet. The water content of the solvent system was about half that of the solvents used by Buzzati-Traverso and Rechnitzer. This reduction of water content appeared to be essential for a clear differentiation between the patterns from the two species (Figs. 2 and 3).

Chromatograms from Leiognathus *spp.* and Caranx *spp.*

A few chromatograms were prepared from *Leiognathus insidiator* (total length, 58–73 mm.), *Leiognathus daura* (t.l., 72–80 mm.), *Caranx leptolepis* (t.l., 141 mm.) and *Caranx sansun* (t.l., 118 mm.). A double run was employed in the ascending chromatograms similar to the double run in the disk chromatograms. Spots characteristic of the pattern from *L. insidiator* were absent in the case of *L. daura* and likewise a spot typical of *C. leptolepis* chromatogram was missing in the *C. sansun* pattern (Figs. 4 and 5).

Measurements of Pattern Spread

The quantity of muscle spotted varied from sample to sample and, for comparing the systematic differences found, the ratios of the spreads of chromatographic patterns were calculated in each experiment in preference to the absolute values. In nine experiments in which the solvent front advanced a distance of 19–21 cm., the lengthwise spread of the patterns in the ascending chromatograms had the following chracteristics: Spread (gibbosa)/spread (albella): range, 1·05 to 1·26; mean, 1·12; s.d., 0·07; and coeff. of variation, 6·3%. In the patterns obtained by the disk method, the ratio, R, of the distance from base to lower edge of bottom spot to the distance from base to upper edge of topmost spot was higher in *S. gibbosa* than in *S. albella*, the values of *R.* (*gibbosa*)/*R.* (*albella*) in three experiments being 1·16, 1·14 and 1·12.

Chromatography of Preserved Muscle

A study was also made of the related question of the chromatography of preserved samples. Preservation of muscle in rectified spirit resulted in loss of some of the ninhydrin-positive spots. Treatment with acetone rendered the muscle too fibrous to be conveniently spotted on the filter-paper. Prolonged drying at room temperature caused a loss of the intensity of the patterns and drying the muscle at 100° C. (*cf.* Ceriotti, 1955) resulted in a distortion of the patterns. The best results with minimum or no distortion were obtained when the muscle was pre-treated with ether overnight. The ether-treated muscle could be pressed on to a filter-paper by applying pressure for a few minutes with a glass rod.

DISCUSSION

The chromatographic patterns described by Buzzati-Traverso and Rechnitzer were obtained from fresh fish-tissue; but the experiments reported here indicate that even when the fish are not quite fresh, similar species-specific patterns can be obtained by suitably modifying the experimental conditions, especially the water content of the irrigating solvent-system. The test samples of muscle can be preserved, if necessary, in

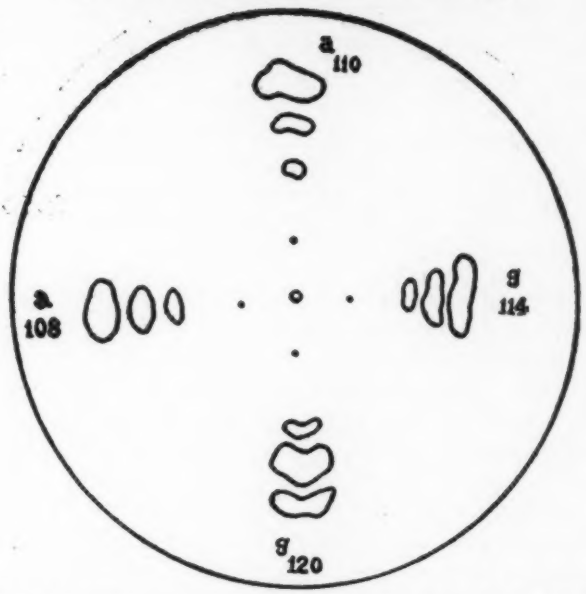

FIG. 3. Ninhydrin-positive patterns of *Sardinella albella* (a) and *Sardinella gibbosa* (g) (Disk chromatography).

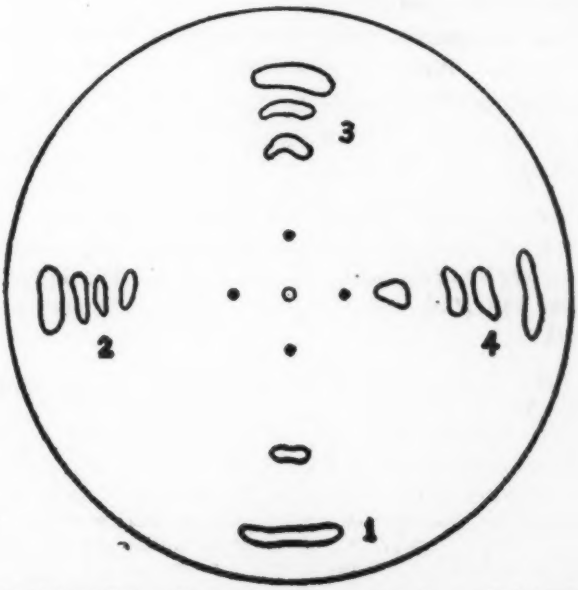

FIG. 5. Ninhydrin-positive patterns of (1) *Leiognathus daura*, (2) *Leiognathus insidiator*, (3) *Caranx sansun*, and (4) *Caranx leptolepis*.

B6

suitable solvents like ether which do not significantly alter the patterns and they can be later chromatographed against standard or authentic specimens and compared. Further, the amount of muscle required in each experiment is only of the order of a few c.mm., and difficulties due to smallness of size of fish are minimised. These features of the method are of obvious advantage in dealing with a large and representative number of samples from commercial catches which are usually of varying degrees of freshness.

The chemical identity of the substances responsible for the specificity of the chromatographic patterns* is not known with definiteness (Buzzati-Traverso and Rechnitzer). This accounts to a large extent for the necessity for standardising *ab initio* the experimental conditions of solvent system, equipment, types and sizes of filter-paper, etc., best suited for the species under comparison. This notwithstanding, the data available suggest the scope for an extensive use of the chromatographic method in standard fishery biology problems of the type enumerated by Kesteven and Deacon (1955). In the sardine fishery near Mandapam, for instance, the bulk of the catches belong to two closely related species (Sekharan, 1955) and basic taxonomic issues are involved in the analysis of fish stocks with reference to their composition, fluctuations in composition from year to year and from area to area, effects of geographical isolation on the stocks, etc. It is in the study of these that paper chromatography with its high degree of specificity is likely to be helpful and even necessary.

SUMMARY

Paper partition chromatography by the ascending and disk methods has been applied to the study of muscle from *Sardinella* spp., *Leiognathus* spp., *Caranx* spp., *Hemirhamphus georgii* and *Atherina* sp.

Experimental conditions have been standardised under which differences among species of fish could be made out qualitatively and on the basis of quantitative measurements, using fish tissue of varying degrees of freshness. Methods have been studied for the preservation of fish muscle for chromatographic work.

The applications of paper chromatography to problems in fishery biology have been indicated.

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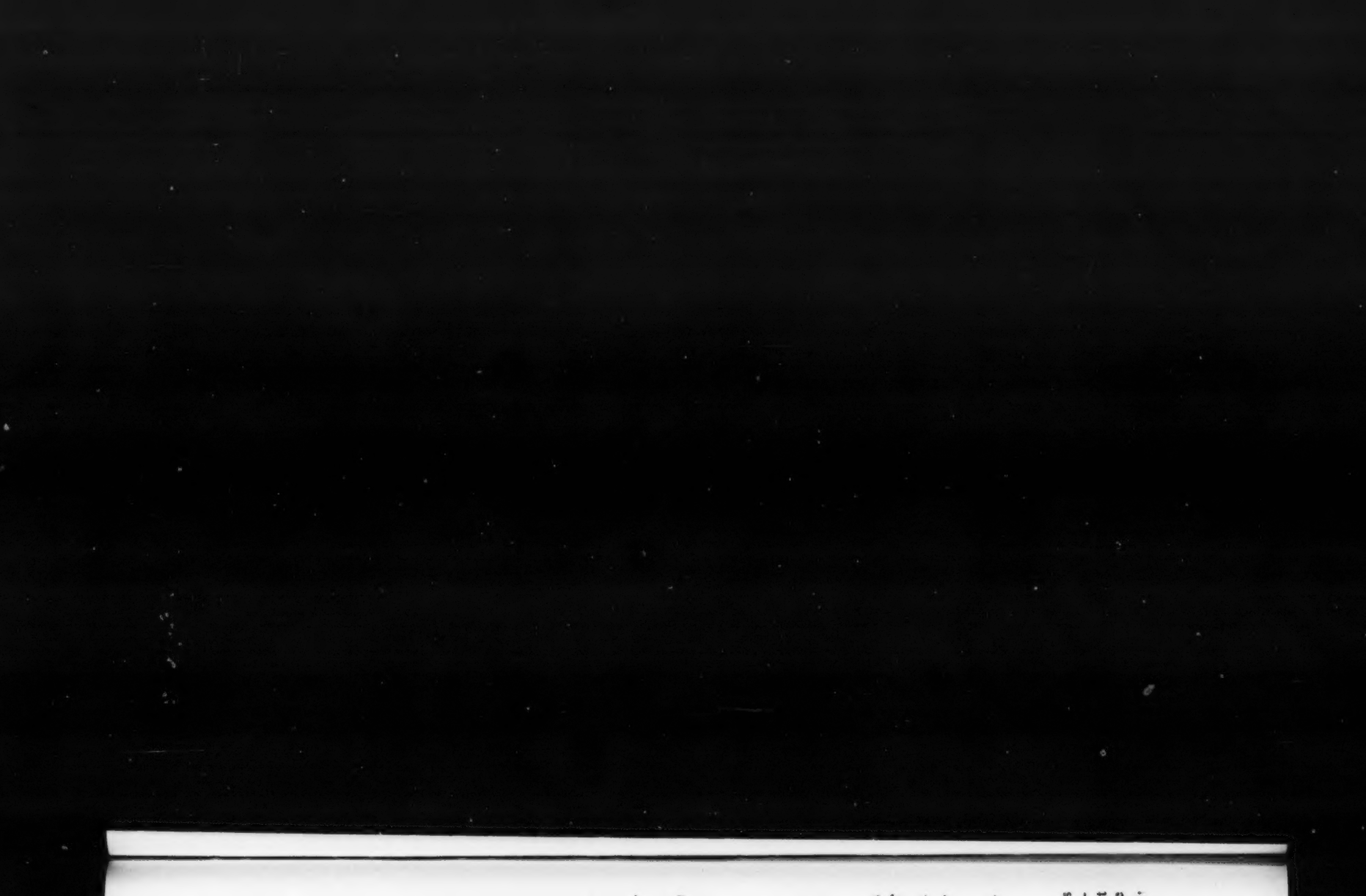
* Very recently Fox (*Science*, 1956, **123**, 143) has been able to detect sex-specific differences (*Drosophila melanogaster*) also by substituting two-dimensional chromatography for the one-dimensional procedure of Buzzati-Traverso and Rechnitzer. His results are of particular interest since they substantiate evidence presented in the present article in regard to the scope of the method in modern taxonomy and to the necessity for a quantitative approach to the problem.



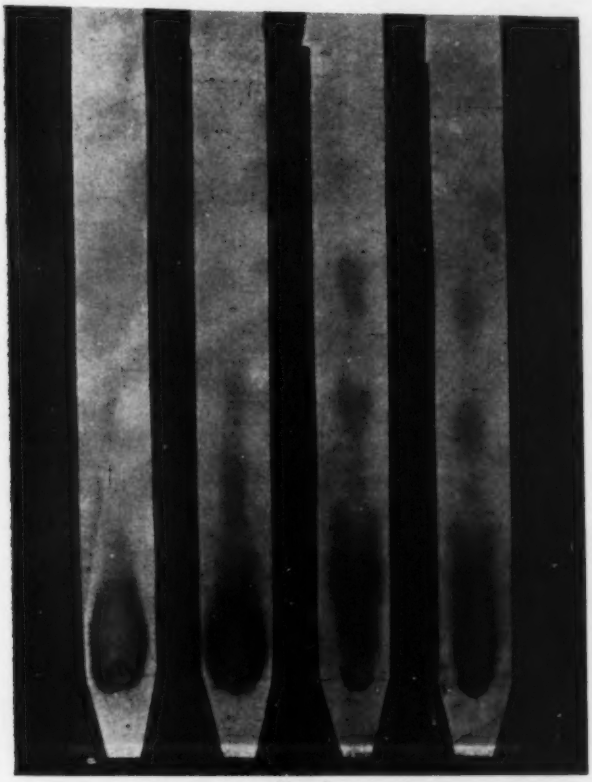


FIG. 1

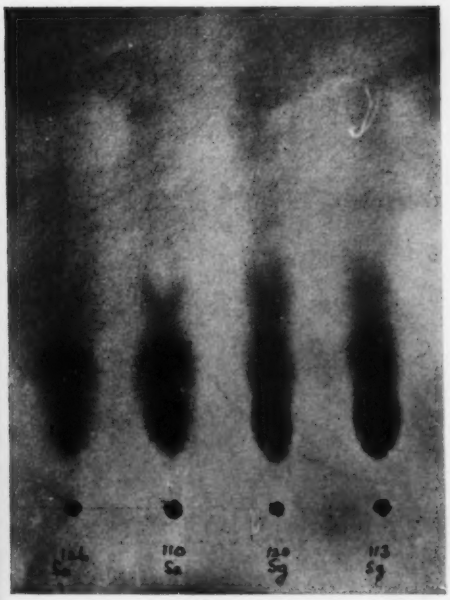


FIG. 2

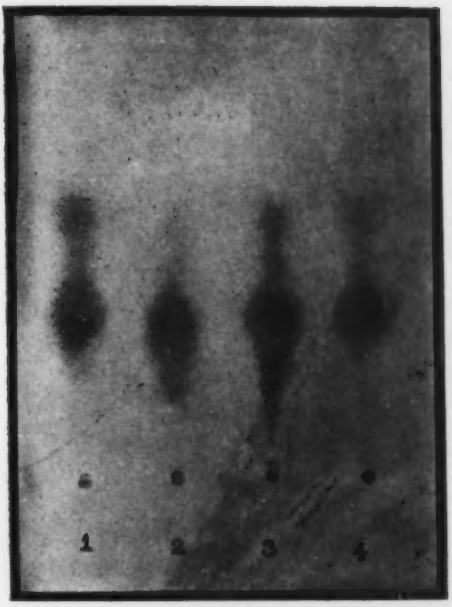


FIG. 4

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REFERENCES

Buzzati-Traverso, A. A. and Rechnitzer, A. B. — "Paper partition chromatography in taxonomic studies," *Science*, 1953, **117**, 58–59.

Buzzati-Traverso, A. A. — "Identification of recessive gene heterozygotes by means of paper partition chromatography," *Nature*, 1953, **171**, 575–76.

Ceriotti, G. .. — "A simple paper chromatographic technique for the study of free metabolites of tissues and body fluids," *Ibid.*, 1955, **175**, 897–98.

Giri, K. V. .. — "Filter-paper disk chromatography," *Ibid.*, 1953, **171**, 1159.

Kesteven, G. L. and Deacon, G. E. R. — "Contributions of oceanographic research to fisheries science," *FAO Fisheries Bull.*, 1955, **8**, 67–76.

Kirk, R. L., Main, A. R. and Beyer, F. G. — "The use of paper partition chromatography for taxonomic studies of land snails," *Biochem. J.*, 1954, **57**, 440–42.

Krishna Pillai, V. .. — "Paper chromatography of sugars by the multiple development (ascending) technique," *J. Sci. Ind. Res.*, 1953, **13 B**, 181–82.

Marine Fishery Research Committee, California — "California co-operative oceanic fisheries investigations: Progress Report, 1953, 1-7-52 to 30-6-53.

Micks, D. W. .. — "Paper chromatography as a tool for mosquito taxonomy: The *Culex pipiens* complex," *Nature*, 1954, **174**, 217–18.

Sekharan, K. V. .. — "Observations on the choodai fishery in the Mandapam area," *Ind. J. Fish.*, 1955, **2**, 113–32.

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